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CONTENTS

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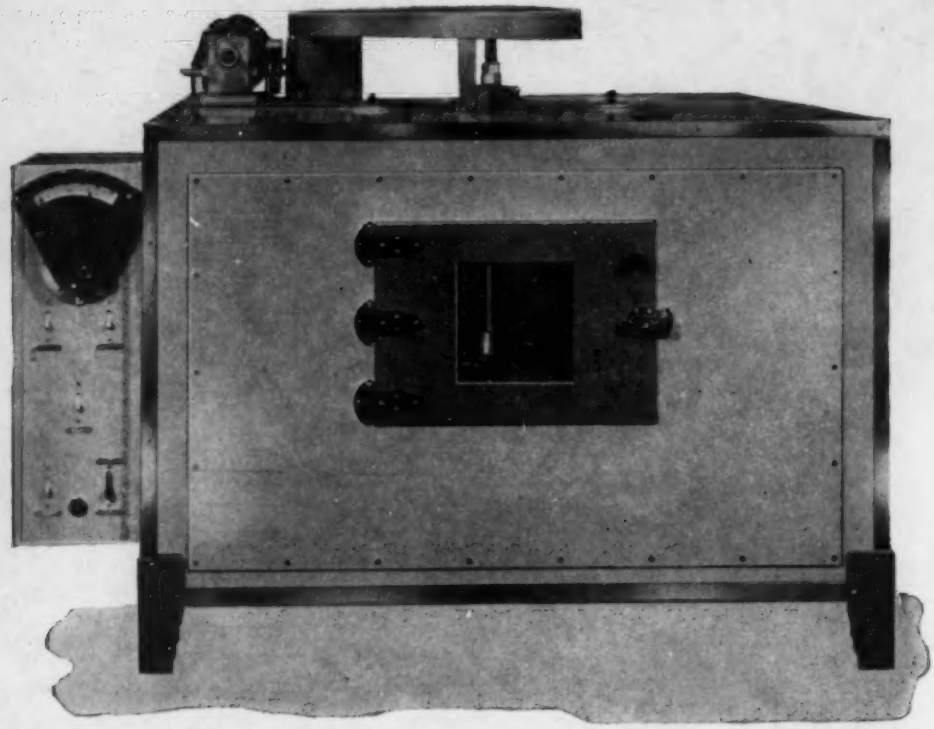

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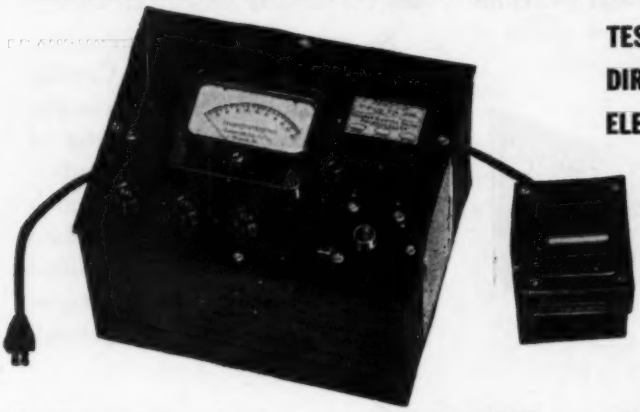

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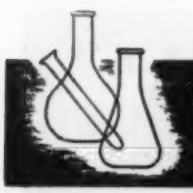

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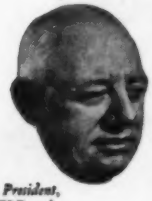

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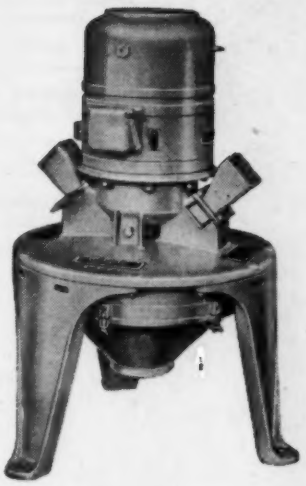

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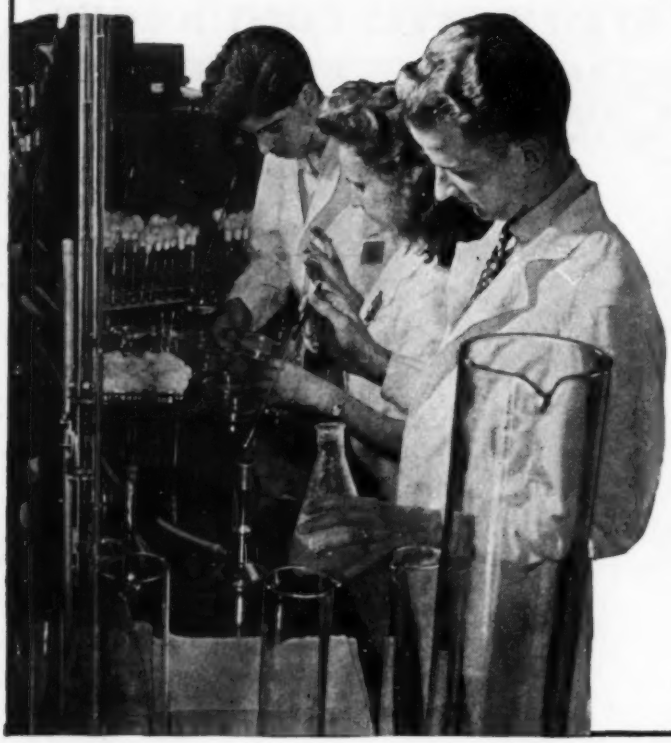

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CEREAL CHEMISTRY

VOL. XXIV MAY, 1947 NO. 3

METHODS FOR DETERMINATION OF ALPHA-AMYLASE. III. IMPROVED STARCH LIQUEFYING METHOD [1]

QUICK LANDIS [2] and SUTTON REDFERN

The Fleischmann Laboratories, Standard Brands Inc., New York, N. Y.

(Received for publication November 23, 1946)

There are two general methods available for specifically determining alpha-amylase: the measurement of the dextrinization time in the presence of an excess of beta-amylase by the method of Sandstedt, Kneen, and Blish (1939), and the liquefaction procedures of Jozsa and Johnston (1935) and Blom and Bak (1938).

Since the liquefying and dextrinizing functions of alpha-amylase are not always equivalent, as shown by Redfern and Landis (1946), it follows that a knowledge of both activities is needed to study and characterize more fully a given alpha-amylase. The Sandstedt, Kneen, and Blish (1939) dextrinizing method with the improved color standard described by Redfern (1946) is a fully satisfactory method. On the other hand, neither of the available liquefying methods is entirely satisfactory. Therefore, an improved starch liquefying method for the determination of alpha-amylase was developed and is herein described. This liquefying method has advantages over the dextrinizing method in that it measures the initial steps in the breakdown of the starch substrate (Hollenbeck and Blish, 1941) and in that for an equivalent reaction time it is about 20 times more sensitive.

The original procedure of Jozsa and Johnston (1935) has never achieved widespread use because of the meticulous care required to prepare a starch substrate with a specified initial viscosity. The pipette type viscometer used in this method is cheap and readily available, but it has the disadvantage of requiring a large quantity of substrate for each determination, viz. 150 g. Because each batch of substrate is limited to 2000 g, more than one batch must be prepared if more than 9 or 10 samples are to be analyzed. In conformity with the

[1] Revised and completed by Sutton Redfern from manuscripts of a paper read by Quick Landis at the Annual Convention, May, 1943.

[2] Deceased.

Lintner method used by the brewing trade in 1935, the method is calibrated for use at 21° C. Such a low temperature is rather difficult to maintain during the summer in many laboratories. A higher temperature of 30°C has been substituted in the improved method; in this respect it conforms with the methods developed by Sandstedt, Kneen, and Blish (1939) and Kneen and Sandstedt (1941) which also use this temperature.

The Blom and Bak (1938) method depends upon observing the time required for the viscosity of a starch paste to change from a viscosity equal to twice the viscosity of a reference solution of sucrose to the same viscosity as the sucrose solution. Since it is impossible to determine these two times by only two measurements, they must be obtained by interpolation from several measurements bracketing each desired viscosity. Unless several viscometers are available, the method is time-consuming. Blom and Bak stated that the results are not influenced by the quality, origin, or age of the starch; intensity of stirring; temperature during the preparation of the starch paste; the viscosity of the paste; or the viscometer. Unpublished data (Landis) showed that the results are definitely influenced by some of these variables.

The new method described herein was developed to overcome the aforementioned disadvantages of the other liquefying methods. The Waring Blendor, which is now readily available, is used for preparing the substrate. A capillary type viscometer is used instead of a pipette. This reduces the quantity of substrate needed for each determination and consequently one batch of substrate is sufficient for about 25 determinations. It was also found that the initial viscosity of the starch paste could vary over a limited range without affecting the results; this simplifies the preparation of the substrate. Only one viscosity reading is necessary since the improved method is a constant time-variable conversion type of method.

Improved Viscometric Method for Determination of Alpha-Amylase Activity

REAGENTS

Acetic acid, 3 N. Dilute 172 ml of C.P. glacial acetic acid to one liter with distilled water.

Sodium hydroxide, 2.25 N. Dissolve 90 g of solid C.P. NaOH in distilled water and dilute to one liter.

Stock diluting solution, 0.025 molar calcium chloride. Dissolve 11.1 g of anhydrous C.P. $CaCl_2$ in distilled water and dilute to 4 liters.

Stock peptizing solution, 2.5% sodium chloride. Dissolve 25 g of C.P. NaCl in distilled water and dilute to one liter.

Starch. Aroostoocrat brand [3] refined potato starch of known moisture content. Other brands of potato starch have not been tested and, therefore, should not be used. The moisture content may be determined by drying a sample of the starch for 2 hours at 130°C in a forced draft oven.

VISCOMETER

The viscometer is a modification of the Ubbelohde (1938) viscometer. A number of laboratory-made viscometers were investigated and it was found that moderate changes in capillary diameter, bulb volume, and driving head had little influence on the viscosity measurements. In the interests of more accurate determinations, however, the dimensions of the viscometer were standardized. Figure 1 is a drawing of the modified Ubbelohde viscometer with the recommended dimensions.[4] The diameter of the capillary is critical and this is made from precision bore tubing. A water jacket is fitted around the viscometer and water from a constant temperature bath at 30°C is circulated through this jacket.

The method of using the viscometer is as follows. The tip of the viscometer is placed in the starch mixture, and the mixture is drawn into the viscometer by holding the finger over the top of the side tube. Both tubes are released simultaneously forming the "suspended" level. The time is measured for the solution to pass between the two reference marks.

For the purpose of standardizing the initial viscosity of the starch paste and calculating the outflow time of fully liquefied starch paste, the viscometer must be calibrated. This is accomplished by measuring the outflow time at 30°C of a 60% (by weight) sucrose solution and then calculating the viscometer constant from the calibration equation:

$$\eta = Ktd \qquad (1)$$

where η = viscosity in centipoises
K = viscometer constant
t = time of outflow
d = density of solution.

The viscosity and density of the sucrose solution are given by Bates (1942) as 34.07 centipoises and 1.284 respectively at 30°C. Unless the sucrose solution is prepared very carefully, it is more accurate to measure the actual concentration of the solution either refractome-

[3] This brand of starch may be purchased from Morningstar Nicol Inc., 650 West 51st Street, New York, N. Y.

[4] This viscometer may be secured from the Ace Glass Company, Vineland, N. J., by specifying Catalog No. V-1039G.

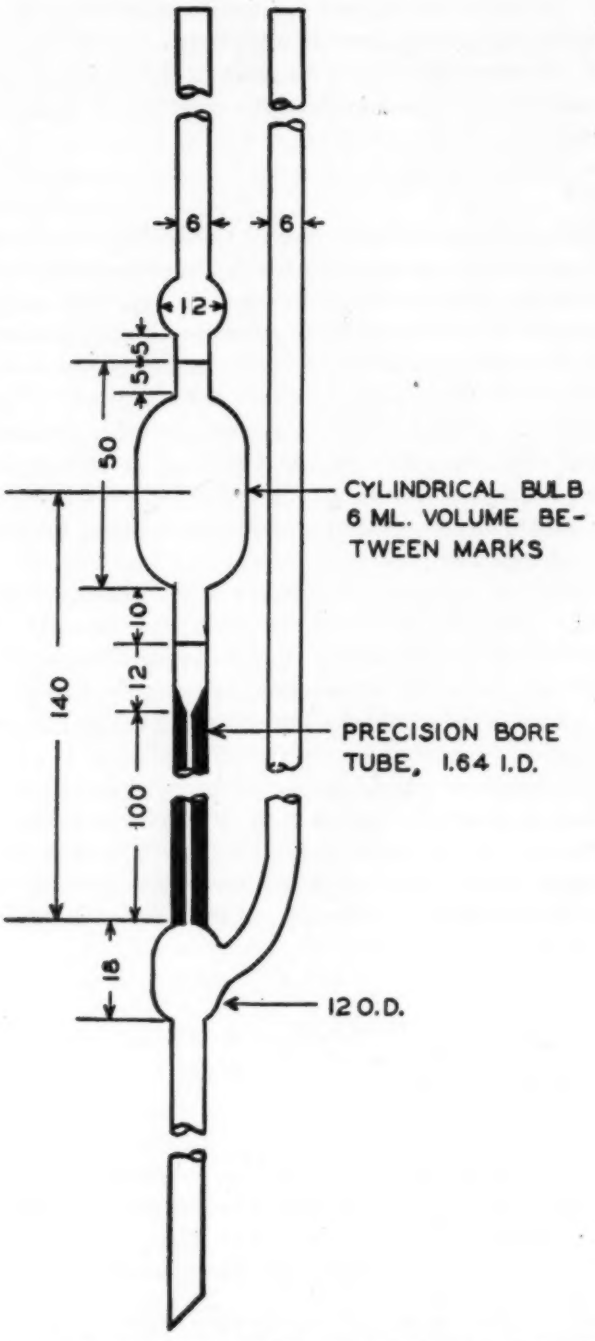

Fig. 1. Drawing of modified Ubbelohde viscometer. (All dimensions are in millimeters.)

trically or densimetrically, since even a 0.1% error in the concentration makes a considerable change in the viscosity. The viscosity corresponding to the actual measured concentration can be found from the tables given by Bates (1942). The dimensions of the viscometer are such that the viscometer constant is approximately 0.4.

PREPARATION OF STANDARD STARCH PASTE

The 5% buffered potato starch paste is prepared with the Waring Blendor in the following manner. Weigh out 42.5 g of starch (dry basis). Fill the tared Blendor jar with tap water at 50°-55°C to warm it to this temperature. Heat 420 ml of distilled water to boiling, and heat 360 ml of distilled water containing 10 ml of 3 *N* acetic acid to 50°-52°C. When the 420 ml portion of water reaches incipient boiling, pour out the water from the warmed Blendor jar, add the starch and the 360 ml of water at 52°C, give the Blendor switch a few flips to bring the starch into suspension, then turn the switch on and pour the 420 ml of boiling water into the vortex of the agitated mixture as rapidly as possible. Start a stopwatch as the mixture gels, which is evidenced by the mixture suddenly becoming extremely thick and translucent in appearance. Police the walls of the Blendor jar with a stirring rod so as to induce the formation of a vortex. After 3.0 minutes of stirring add 10 ml of 2.25 *N* NaOH to neutralize the acid and to buffer the substrate to a pH of 5.2. Continue stirring until 30 seconds short of the total stirring time, as found by previous trial experiments. Make to a net weight of 850 g with water and stir for the additional 30 seconds. Transfer the hot substrate to a one liter Erlenmeyer flask and cool it under the cold water tap to approximately 30°C. Subdivide the substrate into 30 g aliquots by dispensing into 50 ml Erlenmeyer flasks from a one liter separatory funnel. A triple-beam balance sensitive to 1 cg was found to be very convenient and rapid for weighing out the substrate aliquots. Stopper the flasks with clean rubber stoppers and place them in a 30°C water bath to attemperate.

The total time of stirring will vary according to the efficiency of the stirrer, and each operator must adjust his time to obtain a standard paste. A satisfactory paste must have an initial or blank viscosity between 80 and 90 centipoises, when determined as described under PROCEDURE. Total stirring times have been found to vary from 5 to 20 minutes with different Blendors. In case the stirring time with a particular Blendor is less than 5 minutes total, the Blendor can be run at a reduced voltage in order to increase the stirring time to at least 5 minutes, which will allow time for all the necessary steps in preparing the substrate.

PREPARATION OF ENZYME SOLUTIONS

Table I gives the concentration of enzymic materials of various strengths in milligrams per 100 ml of final enzyme solution necessary for proper application of the method.

TABLE I

SAMPLE WEIGHT REQUIRED FOR ANALYSIS OF DIFFERENT PREPARATIONS

Liquefons per g	Sample weight per 100 ml of final dilution
	mg
1–10	1,250
5–50	250
10–100	125
50–500	25
250–2,500	5
1,000–10,000	1.25

In the case of barley malt, 3 to 5 g of finely ground malt is extracted for one hour at 30°C with 100 ml of the 2.5% sodium chloride solution with occasional shaking. The extract is then filtered, the first runs discarded, and the appropriate dilution made with the stock calcium chloride diluting solution.

In the case of diastatic malt syrups, a sufficient quantity is weighed and diluted directly with stock diluting solution. Other enzyme preparations of microbial origin are also diluted directly with the stock diluting solution.

The final enzyme dilution must always be made with calcium chloride diluting solution. Hollenbeck and Blish (1941) showed that calcium ions exert a protective effect on alpha-amylase in dilute solutions. The final dilution is so great that considerable loss of enzyme will take place unless calcium ions are present. In exceptional cases where the calcium chloride solution cannot be used, the final dilutions may be made with 0.25% sodium chloride solution.

PROCEDURE FOR DETERMINATION OF LIQUEFACTION

The starch substrate and enzyme solutions are previously attemperated in a water bath at 30°C. Three ml of enzyme solution is pipetted onto 30 g of starch substrate by flowing the solution gently down the side of the flask so that the enzyme solution overlays the viscous substrate in a distinct layer. The layers are mixed without undue delay by stoppering the flask with a clean rubber stopper and shaking vigorously for 0.1 minute, counting the start of the reaction time from the beginning of shaking. Return the flask to the water bath. After 58 to 59 minutes the mixture is drawn into the viscometer and the outflow time determined. The mixture should pass the upper mark of the viscometer as close to 59 minutes as possible in

order to correct for the liquefaction occurring during the viscosity measurement. The nominal reaction time is one hour. If a succession of samples is being tested, the viscometer is rinsed once with the hydrolyzing mixture before drawing in the aliquot for measurement.

The initial or blank viscosity is determined by adding 3 ml of the stock calcium chloride diluting solution to 30 g of starch substrate and measuring the outflow time after standing for approximately one hour at 30°C. The initial viscosity is calculated from the outflow time by using equation 1 with the appropriate viscometer constant and a value for the density of 1.015. The substrate is satisfactory if the initial viscosity is between 80 and 90 centipoises.

In pipetting the enzyme solution it is necessary to avoid the introduction of traces of saliva. A small cotton plug effectively prevents such contamination.

CALCULATIONS

From the outflow time of a given mixture the percentage decline in viscosity is calculated by the following equation:[5]

$$P = \frac{t_0 - t}{t_0 - t_f} \times 100 \qquad (2)$$

where P = percentage decline in viscosity
t_0 = initial or blank outflow time
t = outflow time of mixture after 60 minutes
t_f = calculated outflow time for fully liquefied starch.

Fully liquefied starch substrate has a viscosity of 0.885 centipoise and a density of 1.015. Using these values and the viscometer content, K, the outflow time t_f for fully liquefied starch is calculated from equation 1.

When the percentage decline is known, the enzyme content per 100 ml of the enzyme solution is obtained by reference to Table III. Knowing the concentration of the solution, the number of liquefons per gram of preparation is calculated. For example: if $t_0 = 215.4$ seconds, $t = 102$ seconds, and $t_f = 2.2$ seconds, then $P = 53.2\%$. From Table II this corresponds to 4.89 liquefons per 100 ml. Each 100 ml of the enzyme solution contained the equivalent of 10 mg of malt, and, therefore, the original malt had an enzyme content of 489 liquefons per gram.

[5] The fundamental equation for calculating the percentage decline in viscosity actually is:

$$P = \frac{\eta_0 - \eta}{\eta_0 - \eta_f} \times 100 \qquad (3)$$

where η_0 = initial or blank viscosity
η = viscosity of mixture after 60 minutes
η_f = viscosity of fully liquefied starch.

However, if the value for each viscosity is replaced by its equivalent from equation 1, it will be seen that the values of K and d being constant cancel out leaving equation 2.

The calibration table may be used for amylase preparations derived from barley malt. It may also be used, although with not quite as high an accuracy, for bacterial alpha-amylase as shown by Redfern and Landis (1946). When used for fungal alpha-amylases, the percentage decline must be limited between 15 and 45%. Within this range the maximum enzyme strength of fungal amylase is obtained.

CALIBRATION OF IMPROVED METHOD

The unit of alpha-amylase, termed a liquefon, is defined by Jozsa and Johnston (1935) as that amount of the enzyme which will convert their specified starch substrate at an initial rate of 25 mg of starch (dry basis) per minute per 110 ml of reacting mixture at 21°C. The new method uses a substrate of higher susceptibility and employs a higher reaction temperature. If we desire to express the enzymic strength of a preparation in terms of liquefons, the new method must be calibrated by means of a sample standardized by the original method. Landis (1945) has discussed the problem of enzyme units, calibration, and standardization.

Rather than actually establishing rate curves and determining the concentration of a standard sample from the original definition of a liquefon, the liquefon content of a selected malt syrup was determined very carefully on several days and at several concentrations by the Jozsa and Johnston method. The average enzyme strength was

TABLE II

CALIBRATION DATA FOR IMPROVED LIQUEFYING METHOD

Weight of enzyme, mg per 100 ml	Percentage decline in viscosity	Average deviation
		%
0.5	3.15	13.3
1.0	7.01	8.4
2.0	14.4	2.8
3.0	21.1	1.6
4.0	27.7	2.0
5.0	33.4	1.8
6.0	38.4	1.8
7.0	42.5	1.6
8.0	46.7	1.5
9.0	50.0	1.5
10.0	53.2	1.7
12.5	59.3	1.4
15.0	63.9	1.6
20.0	69.7	1.0
25.0	74.0	1.1
30.0	77.8	1.0
35.0	80.6	0.7
40.0	82.7	0.8
45.0	84.5	0.8
50.0	86.1	0.8

TABLE III

CALIBRATION TABLE FOR MALT ALPHA-AMYLASE
IMPROVED LIQUEFYING METHOD

Percentage decline in viscosity	Liquefons per 100 ml of enzyme solution	Percentage decline in viscosity	Liquefons per 100 ml of enzyme solution
15	1.025	45	3.699
16	1.098	46	3.831
17	1.171	47	3.967
18	1.244	48	4.104
19	1.318	49	4.246
20	1.391	50	4.392
21	1.464	51	4.543
22	1.537	52	4.699
23	1.610	53	4.860
24	1.684	54	5.031
25	1.757	55	5.217
26	1.830	56	5.402
27	1.903	57	5.597
28	1.976	58	5.802
29	2.054	59	6.022
30	2.133	60	6.256
31	2.216	61	6.505
32	2.303	62	6.769
33	2.396	63	7.056
34	2.489	64	7.369
35	2.586	65	7.710
36	2.684	66	8.081
37	2.786	67	8.486
38	2.889	68	8.926
39	2.996	69	9.399
40	3.104	70	9.906
41	3.216	71	10.44
42	3.333	72	11.00
43	3.450	73	11.59
44	3.572	74	12.20
		75	12.83

found to be 488 ± 14 liquefons per gram. Dilutions of this sample varying from 0.5 to 50 mg per 100 ml were prepared and the percentage decline in viscosity in one hour determined as described under PROCEDURE. Two different lots of potato starch, two viscometers, and varying initial viscosities in a total of 13 different experiments were used without any significant changes being noted in the final result. The experimental data for the calibration are given in Table II. At enzyme concentrations less than 2 mg per 100 ml the average deviation in the percentage decline in viscosity was excessive, and at concentrations over 25 mg the change in viscosity with concentration was considered to be too slow for accurate use. The most suitable range of viscosity decline was chosen as between 15 and 75%.

A suitable empirical equation to represent the data could not be found. Resort was made to a combination of graphical interpolation and tabular smoothing (Worthing and Geffner, 1943) for preparing the final calibration table. The number of liquefons per 100 ml of enzyme solution for each integral percent decline in viscosity is given in Table III.

Summary

An improved starch liquefying method for the determination of alpha-amylase is described. The novel features of this method are the use of the Waring Blendor for preparation of the standard potato starch substrate, and the use of a modified Ubbelohde viscometer for the measurement of viscosity. The method is calibrated for barley malt alpha-amylase in liquefon units.

Acknowledgment

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Literature Cited

Bates, F. J., and Associates.
1942 Polarimetry, Saccharimetry and the Sugars. Circular C440, Washington, U. S. Government Printing Office.

Blom, J., and Bak, A.
1938 Über die Bestimmung von Amylasen durch Verflüssigung von Stärkekleister. Z. physiol. Chem. **256**: 197–207.

Hollenbeck, C. M., and Blish, M. J.
1941 Parallelism between starch dextrinizing and liquefying activities of amylases. Cereal Chem. **18**: 754–771.

Jozsa, S., and Johnston, W. R.
1935 Determination of alpha-amylase. Ind. Eng. Chem. (Anal. Ed.) **7**: 143–146.

Kneen, E., and Sandstedt, R. M.
1941 Beta-amylase activity and its determination in germinated and ungerminated cereals. Cereal Chem. **18**: 237–252.

Landis, Q.
1945 Methods for determination of alpha-amylase. I. Classification of enzyme assay methods, standardization of samples, and calibration of modified procedures. Cereal Chem. **22**: 1–11.

Redfern, S.
1947 Methods for determination of alpha-amylase. IV. A permanent glass end-point color standard for use in the dextrinizing methods. Cereal Chem.: to be published.

——, and Landis, Q.
1946 Methods for determination of alpha-amylase. II. Liquefying and dextrinizing activities of alpha-amylases from different sources. Cereal Chem. **23**: 1–15.

Sandstedt, R. M., Kneen, E., and Blish, M. J.
1939 A standardized Wohlgemuth procedure for alpha-amylase activity. Cereal Chem. **16**: 712–723.

Ubbelohde, L.
1937 The principle of the suspended level. Ind. Eng. Chem. (Anal. Ed.) **9**: 85–90.

Worthing, A. G., and Geffner, J.
1943 Treatment of Experimental Data. John Wiley and Sons, Inc., New York, pp. 6–11.

DEVELOPMENT AND SOLUBILITY OF AMYLASE IN WHEAT KERNELS THROUGHOUT GROWTH AND RIPENING [1]

SIGMUND SCHWIMMER

Enzyme Research Laboratory, Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture, Albany, California

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It is well known that the greater portion of the amylase present in ripe wheat or barley exists in a form that is both inactive and insoluble in water. Part of the bound amylase can be liberated by salts or peptones; all of it is freed by certain reducing agents (Balls and Tucker, 1943), and by proteinases, among which papain is probably the most efficient. It is now shown that the amylase of wheat is completely soluble in water when it first appears in the kernel, but becomes progressively less soluble as the grain matures and ripens. The present paper deals with these changes, as observed from day to day in wheat grown on a small experimental plot for two seasons.

During the growth cycle of wheat the appearance and development of several enzymes, among them amylase, were studied extensively by Bach, Oparin, and Wähner (1927). They found that there was initially a slow development of each enzyme, followed by rapid increase to a climacteric, after which a slow decrease occurred as the kernel underwent extensive dehydration. It is now apparent that (for beta-amylase [2]) this decrease is largely due to the formation of the bound form of the enzyme, which is still present in an inactive, but activatable condition.

The liberation of beta-amylase by salts, reducing agents, and proteinases indicates its association with the protein fraction of the grain, so the development of protein in wheat is highly instructive in this connection. Woodman and Engledow (1934) state that the proteins make their appearance in the following order: salt-soluble proteins, gliadin, and finally glutenin. Presumably water-soluble proteins may be expected at early stages of development. Koblet (1940), in an extensive investigation of the chemical constituents of developing wheat plants, obtained evidence of the existence of a steady state with respect to nonprotein nitrogen compounds during much of the development of the grain (i.e., during the middle period thereof). In this steady state, the elaboration of simple nitrogen compounds is balanced by their transformation into proteins. In the earliest stages of growth the elaboration of simple nitrogen compounds is faster than

[1] Enzyme Research Laboratory Contribution No. 101. Part of this work was done under the Special Research Fund authorized by the Bankhead-Jones Act of June 29, 1935.

[2] Practically all the amylase in mature wheat appears to be beta-amylase.

their transformation; during the late milky stage the reverse is true. Eckerson (1917) had already shown that no "storage protein" (gluten) is formed in the endosperm until dehydration becomes appreciable. That a part of the bound amylase is associated with "storage protein" follows from the findings of Dull and Swanson (1941), who presented experimental evidence that amylase is associated with those proteins which undergo peptization with salts.

In the present paper, the beta-amylase found in wheat kernels at various stages of their development has been divided according to solubility into three parts: amylase soluble in water, amylase insoluble in water but dissolved by dilute (5%) sodium chloride solution, and amylase insoluble in the salt solution but liberated by digestion with papain. Inasmuch as digestion with papain brings practically all of the protein into solution, it seems very likely that the total of active and inactive amylase in the wheat is the sum of these three parts, but there is no direct proof that this is so.

Materials and Methods

An experimental plot 10 by 50 feet at Albany, California, was planted with six rows of wheat during the summer of 1943, and another in the spring of 1944. The plot was irrigated by three trenches, one at either side and one in the middle. The seed wheat was the variety *Baart* grown in 1943 near Stockton. The months during which characteristic changes occurred are shown in Table I. In addition, samples

TABLE I

History of Experimental Wheat

Stage	Dates of change		
	Baart plot I	*Baart* plot II	Montana *Thatcher*
Planting	August, 1944	March, 1945	May, 1944
Blossoming	November, 1944	April, 1945	June, 1944
Stiff dough	February, 1945	July, 1945	August, 1944
Ripe wheat	April, 1945	August, 1945	September, 1944

of *Thatcher* spring wheat grown near Bozeman, Montana, in the summer of 1944 were examined concurrently. Weather conditions for satisfactory ripening of the wheat were not good at the California location,[3] nor were they the same for the two seasons.

[3] Plot I was characterized by absence of rain during prepollination stages followed by intermittent rainfall until the ripening stage. The soil was irrigated before pollination. In contrast, the second experiment received rain only during the prepollination period and was not irrigated. Some rust occurred on both wheats during the late dough stage. The Montana wheat, on the other hand, was grown under optimum conditions. Undoubtedly the differences can also be attributed in part to differences in variety, season, and location. Lamb and Bayfield (1941) have investigated the effect of these factors on yield, weight of ash, and protein. McCalla and Corns (1943) concluded that environment appeared to have a greater influence on starch and protein content of both wheat and barley than the weather did, although both factors contributed to the final values obtained.

Five grams of the wheat (ground well in a mortar if wet, or in a Wiley Mill if dry) were incubated in 50 ml of water for 1 hour at 40°C.[4] The resulting suspension was centrifuged, the residue was washed with 50 ml of water, and the washings were combined with the original supernatant liquid. The residue was then suspended in 50 ml of 5% sodium chloride solution, incubated, centrifuged, and washed as before. The residue from sodium chloride extraction was diluted to 40 ml with a 1% suspension of papaya latex and after incubating for 30 minutes at 30°C, it was again centrifuged and washed as before. The papaya latex used was the so-called "salt paste papain" described by Balls, Lineweaver, and Schwimmer (1940), about one-third of which was sodium chloride. Thus three preparations representing the water-soluble, salt-soluble, and papain-soluble amylase of the wheat were obtained. Tests showed that a second washing did not remove a further appreciable amount of amylase. Quick handling was considered desirable to lessen the chances of the decomposition of originally insoluble enzyme into a soluble form.

The difficulties encountered in any attempt to separate enzymes quantitatively admittedly apply to the foregoing procedure. Some of these are discussed later. The presence of one protein in solution may of course modify the solubility of another. But on the whole the amounts of amylase found in each fraction have been very consistent, and are in no sense quantities of borderline magnitude. In many instances the results have agreed well with others obtained at quite different concentrations of solvents.

The beta-amylase content of each preparation was determined by a modification of the method of Kneen and Sandstedt (1941). Ten milliliters of the samples to be tested were added to 20 ml of a 2% starch solution, pH 4.8 (0.02 *M* acetate buffer), and the mixture was incubated at 30° for 10 minutes. Amylolysis was stopped by adding 10 ml of 0.05 *N* potassium ferricyanide to a 3 ml portion (i.e., one-tenth) of the incubated mixture. The amount of ferricyanide reduced by sugars and dextrins formed during the reaction was determined as directed in the publication of Kneen and Sandstedt (1941). One unit of amylase is here defined as that amount of enzyme which, when present in the total digestion mixture, liberates enough sugar to reduce one milliequivalent of ferricyanide in 10 minutes under the given conditions. What is measured is one-tenth of this amount of sugar. The relation between ferricyanide reduced and amylase units (as present in a water extract of flour) is shown in Figure 1.

[4] Attempts to use a "Waring Blendor" were unsuccessful, because the water solubility of the amylase changed with the time of mixing, and each sample used had a characteristic solubility-time curve. This is not surprising, for gluten suspensions change their properties when similarly stirred.

Total beta-amylase was determined in the same manner by using 50 ml of the papain suspension and 5 g of ground wheat.

Total alpha-amylase of the papain-treated material was determined by the method of Schwimmer (1945).

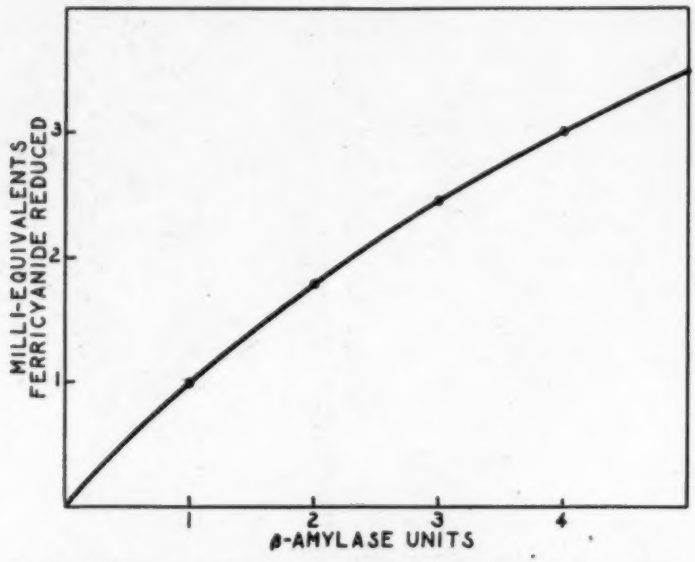

Fig. 1. Relationship between milliequivalents of ferricyanide reduced and units of beta-amylase.

Moisture was determined by heating in air at 115°C for 3 hours; nitrogen by the customary Kjeldahl procedure.

Experimental Results

Weight Changes. Figure 2 depicts the relative weight changes per thousand kernels during the development of various wheat varieties. The wheat from the two *Baart* plots is compared with the data of Woodman and Engledow (1924) for *Red Fife*, and those of Koblet (1940) for *Huron* converted to relative values for ease of comparison. The maximum weight coincides with the soft dough stage of development. Dry weight and nitrogen for normal grain reach an approximate plateau somewhat later (at full development) and remain fairly constant during subsequent ripening. The constancy of nitrogen during the ripening process is an indication that the proteins already present are changing into typical gluten.

Whereas the ripe wheat from plot I approximates the seed wheat with respect to dry weight and nitrogen, these quantities are significantly diminished in the case of the wheat from plot II (Table II), which lost not only water but solids as well. The wheat from *Baart* plot II thus underwent an abnormal change before full maturity was

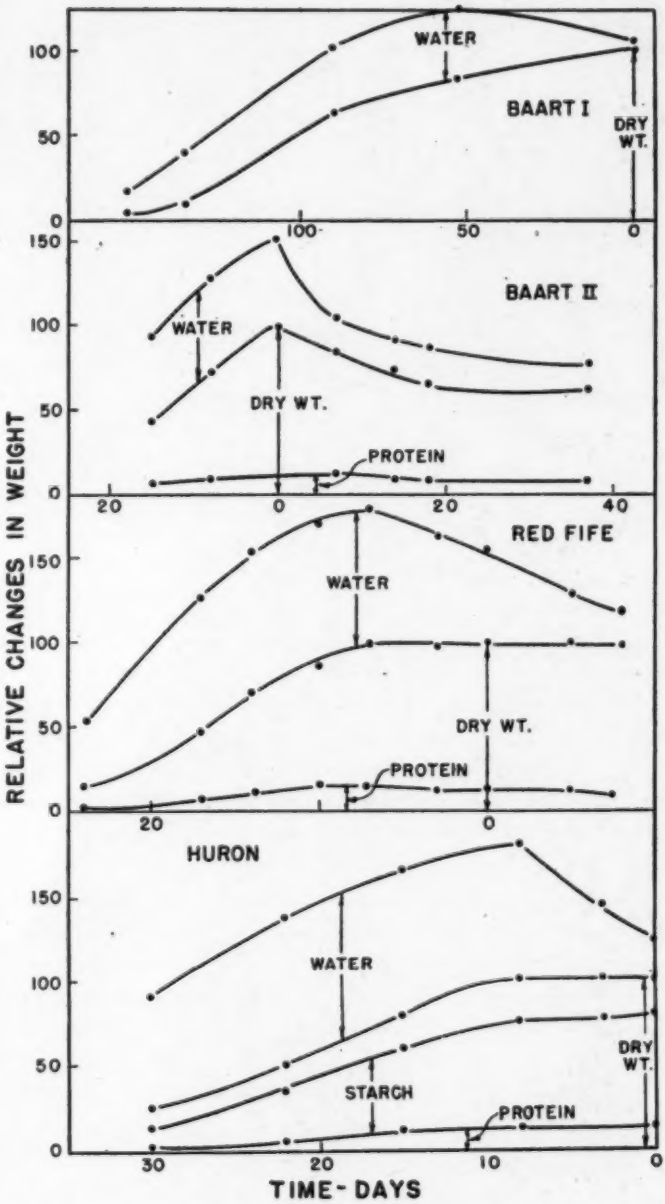

Fig. 2. Changes in weight observed during the development of wheat kernels.

The total weight of the kernel at a given time is of course that of the water plus the dry matter, and is shown by the topmost curve in each set. The total weight is divided, as indicated by the arrows, into the weights of protein, total dry matter, and water. (In the case of *Huron* the weight of starch is also shown.)

The completion of "growth" is taken as the time when the dry weight has reached its maximum. Subsequent changes include "ripening" and loss of water. To simplify comparison with the data of other workers, the maximum dry weight found has been arbitrarily taken as 100 (on the Y axis), while the time has been counted backward or forward from the date on which this maximum occurred.

TABLE II

CHANGES IN WEIGHT, MOISTURE, AND NITROGEN

Wheat and days of harvest after blossoming	Moisture	Nitrogen (dry basis)	Weight per 1,000 grains	Dry weight per 1,000 grains	"Protein" ($N \times 5.7$) per 1,000 grains
	%	%	g	g	g
Seed wheat	9.7	1.70	46	41	4.0
Baart plot I					
24 days	78	—	9	2	—
41 days	75	—	20	5	—
85 days	37	—	51	32	—
116 days	37	—	63	42	—
168 days	8.1	1.43	54	50	4.1
Baart plot II					
14 days	54	2.17	46	21	3.7
21 days	44	2.01	64	36	4.7
29 days	34	2.01	76	50	5.6
36 days	23	1.93	52	40	4.4
45 days	19.0	1.98	46	37	4.15
50 days	14.8	1.93	41	35	3.8
69 days	13.0	2.20	35	31	3.75

attained. There was actually an interval when the rate of decrease during ripening was greater than the rate of increase during development (Table II, Figure 2). It is evident from these data that the loss in weights was largely due to the disappearance of nonnitrogenous constituents. It is believed that the inadequate water supply was responsible for this effect in plot II. Koblet (1940) points out that moisture levels of 40% are critical in wheat with respect to carbohydrate supply.

It can be seen that the weight of water per grain maintains a roughly constant value until maximum dry weight is reached. Thus in the case of *Baart* wheat I, the dry weight increased fivefold, whereas during the same interval the water content remained at a constant level. Similarly, Koblet (1940) found that changes in water content followed changes of nonprotein nitrogen in that the absolute values of both were constant except at the extreme ends of the growing period.

Changes in Beta-Amylase. The solubility of the amylase varies in different wheats. Whereas in most ripe wheats only about a quarter of the enzyme is soluble in water, *Baart* and other varieties grown in California are distinguished by higher solubilities, often 50 to 60% (Table III).[5] The *relative* degree of solubility does not seem referable to the size of the flour particles, and thus to the thoroughness of extraction. Two-thirds of the total activity in a sample of granular flour was

[5] We wish to thank Drs. J. P. Conrad and C. A. Suneson of the Department of Agronomy, University of California, Davis, for the supply of these wheat samples.

TABLE III

CONTENT AND SOLUBILITY OF BETA-AMYLASE IN SOME WESTERN WHEAT VARIETIES (1945)

Variety	Dry weight per 1,000 grains	Beta-amylase per 1,000 grains	Beta-amylase per gram (dry basis)	Nitrogen (dry basis)	Percent amylase soluble in:		
					Water	Salt	Papain
	g	*units*	*units*	%			
Baart	47	614 × 10	133	1.98	58	23	19
Bunyip	45	304	69	2.25	75	17	8
White Federation	43	534	123	2.07	55	10	35
Pacific Bluestem	42	276	66	2.00	58	10	32
Sonora	35	298	87	2.10	55	9	36
Poso 42	30	294	98	2.18	52	12	36

lost by grinding the flour in a ball-mill, but the proportion of the remaining enzyme that was soluble in neither salt nor water was the same as before grinding (Table IV).

TABLE IV

BETA-AMYLASE SOLUBILITIES OF SOME WHEATS AND FLOURS

Wheat or flour	Beta-amylase[1] content	Percent amylase soluble in		
		Water	Salt	Papain
High protein patent	126	15	16	69
Iowa granular	134	22	20	58
Iowa granular (ball-milled)	47	29	13	58
Kansas patent	81	26	28	46
Thatcher wheat	130	25	25	50
Baart seed wheat	127	57	21	22
Baart plot I	121	58	26	16
Baart plot II	106	66	18	16

[1] Units of beta-amylase per gram dry weight.

The development of amylase in wheat is characterized by a steep rise over a relatively short period, as shown in Figure 3 for the increase of amylase per 1,000 kernels, and in Table V on a dry weight or "concentration" basis. The change in kernel weights is shown in Figure 2. The development of enzyme may be illustrated by comparing the results for plot I on the 41st and 85th days after blossoming, the interval between these days being that of rapid increase in enzyme concentration. During this period the average weight of a kernel increased about 8 times, while the amylase content per kernel increased 40 times. Thus the "concentration" of amylase per gram of dry wheat tissue underwent an increase of about fivefold.[6]

[6] Bernstein (1943), who investigated the relation between amylase and starch in developing maize endosperm, found a positive correlation between beta-amylase activity and sucrose content. However, the rate of starch formation seemed to vary inversely with respect to beta-amylase activity, and no statistically significant relations between these two factors were found.

It is evident that almost all of the amylase of the plant is elaborated before the maximum proportion becomes insoluble in water (Table V and Figure 3). The insolubility of the amylase is probably a consequence of the dehydration of the kernel, concomitant with its ripening. It seems as though the amylase present in the kernel is distributed among the water-soluble proteins, salt-soluble proteins, gliadin, and finally glutenin, as these proteins develop successively in the seed.

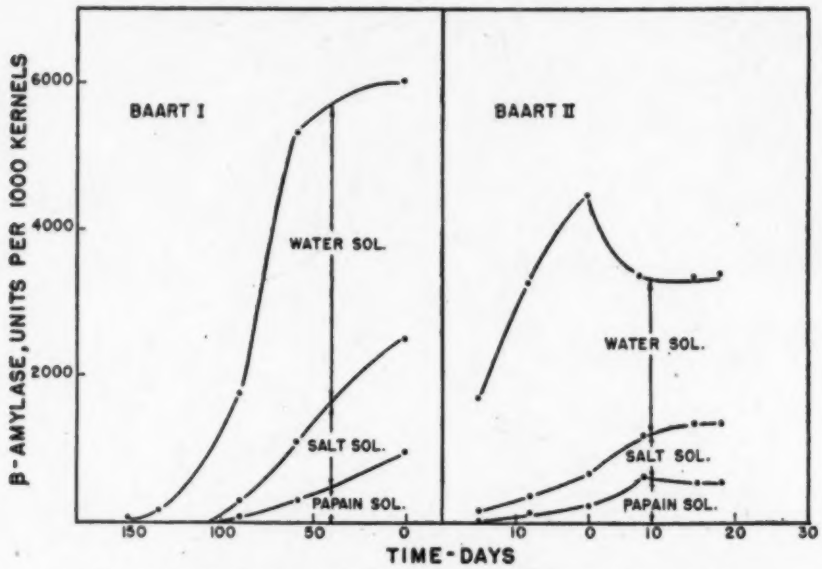

Fig. 3. Development and solubility of beta-amylase of *Baart* wheat samples. The time basis is the same as that used in Figure 2.

It should also be considered that the concentration of proteinase, reducing groups, and salts during the development would also affect these solubility relationships, as determinable in the laboratory, for their presence may alter the observed solubility of amylase protein. Bach, Oparin, and Wähner (1927) measured water-soluble proteinase and amylase in developing wheat. They found that proteinase in the entire kernel did not start to decrease until long after maximum development of amylase and of dry weight had occurred. Since a large part of wheat proteinase is associated with the bran (Balls and Hale, 1936), it is difficult from these data alone to assign a solvent function to the proteinase. Koblet (1940) found that the proteinase content of the wheat endosperm decreases very rapidly when the milk stage of growth has been reached. This is the stage at which considerable amylase starts to undergo solubility changes.

Changes in Alpha-Amylase. The small amount of alpha-amylase per kernel appears to remain relatively constant as compared with beta-

TABLE V

CHANGES IN CONTENT AND SOLUBILITY OF BETA-AMYLASE

Wheat and time of harvest (days after blossoming)	Beta-amylase[1]	Percent amylase soluble in		
		Water	Salt	Papain
Seed wheat	127	57	21	22
Baart plot I				
24	22	100	0	0
41	25	100	0	0
85	55	83	12	5
116	127	79	15	6
168	120	58	26	16
Baart plot II				
14	80	90	8	2
21	91	86	10	4
29	89	85	10	5
36	84	64	16	20
45	90	59	21	20
50	97	62	22	16
69	106	66	18	16
Montana Thatcher				
Early milk stage	121	100	0	
Late milk stage	115	66	34	
Soft dough stage	120	35	65	
Stiff dough stage	127	28	72	
Ripe wheat	130	25	25	50

[1] Units of beta-amylase per gram dry weight.

amylase throughout the entire course of development and early stages of ripening (Table VI). Since the solid matter per kernel is low at the very early stages of growth, the concentration of alpha-amylase is relatively high. Thus the alpha-amylase per gram of dry matter at the very early stage is 68 times that of wheat at complete maturity (i.e., it is about 5 to 10% of that found in germinated wheat), whereas the ratio of beta- to alpha-amylase (using the units employed in this

TABLE VI

ALPHA-AMYLASE IN DEVELOPING WHEAT OF BAART I

Time of harvest (days after blossoming)	Alpha-amylase content $\times 10^4$	
	per 1,000 kernels	per gram (dry wt.)
24	27	13.5
41	26	5.2
85	32	1.0
116	28	0.7
168	10	0.2

paper) is about 6,000 in the ripe wheat; this ratio is lowered to about 17 in very green wheat, a proportion comparable to that found in sprouted grain (i.e., in malt where the ratio is usually 3–10). This may explain the conclusions of Chrzaszcz and Janicki (1936) that the amylase of immature wheat was similar to that of malted barley.

Effect of Changing Water Content. Kneen, Miller, and Sandstedt (1942) have shown that the amount and proportion of total and bound amylase of mature wheat or barley is not affected by the usual soaking process prior to germination. Table VII contains data which confirm

TABLE VII

EFFECT OF CHANGING WATER CONTENT ON BETA-AMYLASE SOLUBILITY
(Baart Wheat Plot II)

Time of harvest (days after blossoming)	Moisture[1]	Percent amylase soluble in		
		Water	Salt	Papain
	%			
14	54	90	8	2
	9.8	43	27	30
21	44	86	10	4
	14.0	50	33	17
29	34	85	10	5
	10.0	60	30	10
69	13.0	66	18	16
	39	66	20	14

[1] The upper row of figures represents values of original sample, the lower row values of the dried or water-soaked sample.

this observation on ripe wheat. On the other hand, drying of immature kernels at 33°C *in vacuo* profoundly affected the solubility relationships of the amylase. Evidently ripening from the standpoint of the withdrawal of water and the effect of such dehydration on the solubility of the amylase is an irreversible process. The younger the plant the greater does artificial change in moisture affect the solubility of amylase. As shown in Table VII, when very young wheat was dried, about half of the previously water-soluble enzyme became insoluble, but much of it still remained soluble in salt solution. This seems to indicate that the appearance of the gluten proteins with time, although a factor of importance in modifying amylase solubility, is not the only factor involved. Whether or not the proteins are separated from each other within the kernel tissues may also be of importance.

The amylase-dissolving effect of any proteinase present must also be considered, particularly because an extract of green wheat is able to

liberate bound amylase from ripe wheat. Thus when green wheat (containing 15% bound amylase) was ground and steeped in water for 1 hour at 40° together with mature wheat (containing 65% bound amylase), the water-soluble amylase of the resulting suspension was about a third higher than the additive values of the two wheats acting separately.

Kernel Size and Total Beta-Amylase. By means of an appropriate series of sieves, the kernels of a bushel of mature *Baart* wheat were separated into four fractions of varying sizes, designated as shriveled, small, medium, and large. The two intermediate fractions constituted by far the major portion of the wheat. The results of total beta-amylase and nitrogen determinations are shown in Table VIII. It can

TABLE VIII

TOTAL BETA-AMYLASE AND NITROGEN OF BAART WHEAT FRACTIONS

Fraction	Shriveled	Small	Medium	Large
Per unit weight				
Dry matter (%)	89.4	89.3	89.2	89.4
Nitrogen (%)	2.46	1.73	1.73	1.78
Beta-amylase $\left(\frac{\text{Units}}{\text{Gram}}\right)$	227	127	127	133
Per 1,000 kernels				
Dry matter (gram)	11.3	24.6	38.0	46.8
Nitrogen (gram)	0.28	0.43	0.66	0.83
Beta-amylase (units × 10)	257	308	480	623
Percent beta-amylase soluble in:				
Water	70	59	56	53
Salt	12	16	14	14
Papain	18	25	30	33

be seen that the amylase and nitrogen contents of the kernels of the *intermediate* fractions were directly proportional to the weight of the kernels. In the smallest kernels, amylase and nitrogen were present in a proportionally higher concentration. This is probably due to the loss of nonnitrogenous dry matter. The greater water-solubility of the amylase of this fraction can be attributed to the high proportion of immature kernels, which presumably would have a higher proteolytic activity. The "large" fraction exhibits a small, but what is believed to be a definite, increase in amylase over the intermediate sizes. This is possibly due to an unusually large proportion of metabolically inert kernels in this fraction.

Summary

The beta-amylase content of *Baart* wheat was followed throughout the development and ripening of the plant. The development of the

beta-amylase within the kernel is characterized by a steep rise over a short period, the rate of increase being of the order of five times the rate of increase in weight over the same period. The apparent decrease in beta-amylase with ripening has been shown to be due to the increasing insolubility in water and in salt solution.

Almost all of the beta-amylase is elaborated before the maximum insolubility of the enzyme occurs. This insolubility is probably a consequence of the dehydration and occurs concurrently with the formation of the final wheat proteins during ripening. These solubility relationships change with artificial moisture changes in the case of immature kernels, but not of mature kernels.

Alpha-amylase is present in about the same amount per kernel throughout growth and development.

Except for very large or very small kernels, the beta-amylase content of the kernels is directly proportional to their weight. Shriveled kernels display a disproportionally higher amylase concentration.

Literature Cited

Bach, A., Oparin, A., and Wähner, R.
1927 Untersuchungen über den Fermentgehalt von reifenden, ruhenden und keimenden Weizensamen. Biochem. Z. **180**: 363–370.
Balls, A. K., and Hale, W. S.
1936 Proteolytic enzymes of flour. Cereal Chem. **13**: 54–60.
——, Lineweaver, H., and Schwimmer, S.
1940 Drying of papaya latex. Stability of papain. Ind. Eng. Chem. **32**: 1277–1279.
——, and Tucker, I. W.
1943 The extraction of diastase and recovery of protein from wheat. Fruit Prod. J. **23**: 15–16.
Bernstein, L.
1943 Amylases and carbohydrates in developing maize endosperm. Am. J. Botany **30**: 517–526.
Chrzaszcz, T., and Janicki, J.
1936 Amylase during the growth and ripening of grains. Biochem. J. **30**: 1298–1302.
Dull, M. F., and Swanson, R. G.
1941 The nature of the liberation of bound barley amylase as effected by salt solutions. Cereal Chem. **18**: 113–120.
Eckerson, S. H.
1917 Microchemical studies in the progressive development of the wheat plant. Washington Agr. Exp t. Sta. Bull. 139.
Kneen, E., Miller, B. S., and Sar dstedt, R. M.
1942 The influence of temperature on the development of amylase in germinating wheat. Cereal Chem. **19**: 11–27.
——, and Sandstedt, R. M.
1941 Beta-amylase activity and its determination in germinated and ungerminated cereals. Cereal Chem. **18**: 237–252.
Koblet, R.
1940 Untersuchungen über die stofflichen Veränderung im wachsenden und reifenden Weizenkorn. Schweiz. botan. Ges. **50**: 99–232.
Lamb, C. A., and Bayfield, E. G.
1941 The influence of season and location on the grain of several wheat varieties. J. Am. Soc. Agron. **33**: 294–303.
McCalla, A. G., and Corns, W. G.
1943 Effects of variety and environment on the starch content of wheat and barley. Can. J. Research **21C**: 307–321.

Schwimmer, S.
1945 The role of maltase in the enzymolysis of raw starch. J. Biol. Chem. **161**: 219–234.
Woodman, H. E., and Engledow, F. L.
1924 A chemical study of the development of the wheat grain. J. Agr. Sci. **14**: 563–586.

CAPRYLIC ALCOHOL—A CAUSE OF VARIATION IN THE FLOUR VISCOSITY TEST

PAUL W. HODLER[1] and ROSEMARY C. SEBASTIANI

The Kroger Food Foundation, Cincinnati, Ohio

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Much of the previous work on the Apparent Viscosity Test (*Cereal Laboratory Methods*, 4th Edition) has been designed toward eliminating variations in results between duplicate tests and between laboratories.

Reiman (1934) recommended a method for standardizing lactic acid and also the use of a MacMichael Viscosimeter operated at 12 r.p.m. He further recommended the use of a No. 30 wire. Bayfield (1934) found that a one-hour digestion period tended to eliminate the influence of flour granulation and demonstrated that machine mixing was preferable to hand mixing. He also pointed out the effect of the quantity and composition of the ash and recommended that the test be made on the basis of a constant weight of protein.

Later, Bayfield (1936) detailed a method for the test including the "no time" method as well as modifications based on a one-hour digestion and a weight of flour calculated to give 2 g of protein in each test.

Harrel (1937) recommended that the flour suspension be prepared by shaking the flour and water in a 500 ml Erlenmeyer flask, as originally suggested by Bayfield. He found that an error of plus or minus 1% in determining the moisture content of flour has very little effect upon the apparent viscosity; on the contrary, a variation of 1 r.p.m. in bowl speed has a marked effect on apparent viscosity.

The results obtained with this test on the Cincinnati-Central States Referee samples showed rather wide variations between laboratories. In an investigation of one laboratory's results it was noted that there was a tendency for the results of successive tests to increase progressively over a period of several months, and the final results were definitely out of line with those of other collaborators.

A careful investigation of the technique, equipment, and reagents showed that this gradual rise was due to a progressive deterioration of

[1] Present address: The Kroger Company, Manufacturing Division, Production Laboratory, Cincinnati 4, Ohio.

the caprylic alcohol. The situation was remedied by purchasing a new supply of caprylic alcohol.

On another occasion, it was impossible to remedy the condition by the purchase of new alcohol since purchases from two separate sources also produced high results. Consequently, a third source was used temporarily with good results. This alcohol was sold under the name of octyl alcohol. When satisfactory caprylic alcohol again was available from the original supplier, it was used.

By the end of two years this lot of caprylic alcohol had so deteriorated as to give results which were 5 to 13 degrees higher than with the use of new alcohol. Typical results with three operators are shown in Table I.

TABLE I

EFFECT OF AGE OF CAPRYLIC ALCOHOL ON APPARENT VISCOSITY RESULTS, DEGREES MACMICHAEL

Flour	New caprylic				Old caprylic[1]			
	Operator			Average	Operator			Average
	1	2	3		1	2	3	
A	27	28	31	28.7	32	34	39	35.0
B	60	61	—	60.5	65	67	—	66.0
C	—	60	54	57.0	—	73	64	68.5
D	—	25	26	25.5	—	35	37	36.0

[1] Two years old.

A test of the so-called octyl alcohol which had stood unused on the laboratory shelf for more than two years showed results to be very little higher than with the new supply of caprylic alcohol. This comparison on the same flours is shown in Table II.

TABLE II

COMPARATIVE FLOUR VISCOSITY DATA EMPLOYING NEW CAPRYLIC ALCOHOL AND OLD "OCTYL" ALCOHOL

Flour	New caprylic				Old octyl[1]			
	Operator			Average	Operator			Average
	1	2	3		1	2	3	
A	27	38	31	28.7	28	31	33	30.7
B	60	61	—	60.5	65	58	—	61.5
C	—	60	54	57.0	61	58	—	59.5
D	—	25	26	25.5	28	28	—	28.0

[1] Over two years old.

Since these data indicate that the octyl alcohol was more stable toward deterioration than the other samples, an effort was made to determine wherein they differed. This investigation was abandoned because of the incomplete history of the samples. It was learned that both caprylic alcohol and octyl alcohol are incomplete chemical designations. Either name might refer to any one of the following, or to a mixture of the three:

(a) Primary normal octyl alcohol
(c) Secondary normal octyl alcohol
(d) 2 ethyl hexyl alcohol

As the exact form of caprylic or octyl alcohol is not specified by *Cereal Laboratory Methods* this may be one cause of laboratory-to-laboratory variation in results.

In view of the wide discrepancies encountered, further work appears necessary to determine whether one form of octyl (or caprylic) alcohol is more stable than another, what types and kinds of minute impurities are likely to be present because of differences in raw materials or processing, whether there is an increase in acidity of the alcohol during aging, and, if so, whether acidity can be used to select a satisfactory octyl alcohol for this purpose.

Summary

Deterioration of caprylic alcohol, used as an antifoaming agent, results in an increase in the apparent viscosity values of wheat flour suspensions. Different samples vary in stability and the source and condition of the caprylic alcohol appears to be one of the uncontrolled factors causing poor agreement between laboratories in the apparent viscosity test.

Literature Cited

Bayfield, E. G.
1934 The viscosity test. Cereal Chem. **11**: 637–647.
1936 Report of the subcommittee on viscosity. Cereal Chem. **13**: 773–788.
Harrel, C. G.
1937 Report on viscosity of flour. Standardization of the MacMichael viscosimeter. J. Assoc. Official Agr. Chem. **20**: 373–382.
Reiman, W.
1934 Methods for determining the viscosity of flour-in-water suspensions. Cereal Chem. **11**: 299–312.

GRAIN STORAGE STUDIES. VI. WHEAT RESPIRATION IN RELATION TO MOISTURE CONTENT, MOLD GROWTH, CHEMICAL DETERIORATION, AND HEATING [1]

MAX MILNER,[2] CLYDE M. CHRISTENSEN,[3] and W. F. GEDDES [4]

University Farm, St. Paul, Minnesota

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Molds were shown by Milner and Geddes (1945, 1946, 1946a) to be primarily responsible for the respiration, heating, and chemical deterioration of soybeans stored at moisture contents encountered in commercial practice. They found that the so-called "critical" moisture content of soybeans, where respiration increases rapidly and the temperature of the stored seeds begins to rise, corresponds to the minimum relative humidity at which molds normally present on and in the seeds begin to grow. They emphasized the fact that maximum respiration in laboratory tests with moist grain can be measured only by using continuous and adequate aeration, and they indicated the utility of the respiratory quotient in evaluating the results of such studies.

In the present studies the respiration and heating of wheat have been investigated, using techniques similar to those employed with soybeans, except that in the present work, molds were assayed quantitatively.

Materials and Methods

A sound sample of Regent wheat, a hard red spring variety, grown at McIntosh, South Dakota, in 1944, testing 94% germination, was used in these studies. Respiratory rates and respiratory quotients exhibited by this wheat under various conditions were determined using the technique described by Milner and Geddes (1945), whereby the seed samples, maintained in respirometer bottles in a thermostat at 30°C, were aspirated continuously with air at a relative humidity which was in hygroscopic equilibrium with the initial moisture content of the samples. The accumulated effluent air was analyzed daily for oxygen and carbon dioxide. Moisture was determined by the two-stage method, and fat acidity and sugars were determined according

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[2] Research Associate, Division of Agricultural Biochemistry, University of Minnesota, St. Paul, Minnesota. Present address: Department of Milling Industry, Kansas State College, Manhattan, Kansas.

[3] Assistant Professor, Division of Plant Pathology and Botany, University of Minnesota, St. Paul, Minnesota.

[4] Professor of Agricultural Biochemistry, University of Minnesota, St. Paul, Minnesota.

to the procedures outlined for wheat in *Cereal Laboratory Methods* (1941).

The degree of internal contamination of the wheat by molds was determined by surface disinfecting the seed with a one-minute dip in a 1% solution of sodium hypochlorite, followed by a brief rinse in sterile water. Fifty seeds surface-disinfected in this way were placed on Smith Humfeld agar, incubated at 30°C, and examined several times during the course of 10 days. Strips of pericarp were also removed from 15 surface-disinfected seeds with sterile forceps, and cultured on hanging drops in van Tiegham cells on potato dextrose agar. Similar strips of pericarp were removed from 20 seeds that had been soaked in water for one hour, but were not surface disinfected. These strips were placed on slides, with the inner side of the pericarp uppermost, stained with methylene blue, and examined microscopically for fungus mycelium.

The total mold population of the various wheat samples used for respiration studies was determined at the end of the tests. A small lot was ground in an intermediate Wiley mill equipped with a 40-mesh sieve. The mill was first flushed with alcohol to disinfect it, and the air-dried samples were ground in succession, beginning with the one at the lowest moisture content and progressing to the highest. The meals so obtained were cultured at various dilutions in malt-salt agar according to the technique described by Christensen (1946).

In one of the respiration trials, the number of surface molds per seed was determined by shaking 100 seeds in sterile water containing one part of Ultrawet [5] in 10,000 parts of water. The spore suspension was then diluted and cultured in malt-salt agar.

Influence of Moisture Content on Wheat Respiration

Respiration trials at 30°C were conducted on Regent wheat at various moisture values. One experiment covered a moisture range of 12.3 to 16.3% with intermediate increments, over a time interval of 20 days; the other trial involved moisture increments from 16.8 to 38.6% for a period of 17 days.

The daily respiratory rates are given in Table I and a graphical presentation of the data showing the influence of moisture content on respiratory rate for the lower moisture range appears in Figure 1.

The maximum interseed carbon dioxide concentration attained in the first experiment, comprising samples with moisture values up to 16.3%, was 3.96%. In the higher moisture range (16.8 to 38.6%) the interseed carbon dioxide concentrations on the final day of the

[5] An aromatic monosodium sulfonate manufactured by Atlantic Refining Company, Philadelphia, Pa.

TABLE I

INFLUENCE OF MOISTURE CONTENT AND TIME ON THE RESPIRATORY RATE OF WHEAT AT 30°C

Respiratory Rate, Mg CO_2 per 100 g Dry Matter Weight Per 24 Hours

Day	Moisture content, %					
	12.3	13.6	13.8	14.5	15.4	16.3
1	0.05	0.13	0.13	0.16	0.20	0.54
2	0.04	0.15	0.18	0.25	0.37	0.86
3	0.06	0.15	0.25	0.31	0.45	1.03
4	0.07	—	—	—	—	—
5	0.08	0.14	0.25	0.36	0.48	1.24
6	0.08	0.15	0.25	0.36	0.51	1.38
7	0.08	0.12	0.26	0.35	0.49	1.72
8	0.09	0.15	0.24	0.36	0.49	2.26
9	0.08	0.12	0.26	0.36	0.53	5.58
10	0.09	0.14	0.23	0.36	0.55	6.98
11	0.10	0.14	0.25	0.33	0.60	—
12	0.04	0.12	0.23	0.33	0.65	15.88
13	0.07	0.10	0.22	0.35	0.78	17.71
14	0.08	0.14	0.23	0.34	0.90	19.21
15	0.07	0.14	0.24	0.36	1.06	20.04
16	0.08	0.13	0.22	0.41	1.25	20.54
17	0.07	0.13	0.22	0.42	1.48	21.06
18	0.07	0.11	0.23	0.46	1.75	21.47
19	0.10	0.14	0.24	0.53	2.08	22.67
20	0.07	0.11	0.23	0.57	2.53	23.35

Day	Moisture content, %					
	16.8	18.5	20.8	25.2	30.5	38.6
1	—	13.8	34.3	166.7	451.0	843.8
2	1.8	13.1	36.8	180.2	365.1	835.7
3	1.4	14.1	56.6	221.3	316.0	891.6
4	1.5	21.0	90.7	282.7	305.8	1150.9
5	1.8	43.6	101.3	349.8	314.0	1638.8
6	1.8	57.5	122.6	420.2	331.4	2170.5
7	2.7	63.4	151.7	510.4	352.4	2509.1
8	4.0	68.9	179.6	596.1	373.8	2685.0
9	5.9	74.5	205.4	674.4	393.7	2754.1
10	9.1	82.2	242.8	757.8	455.7	2834.5
11	12.4	87.9	276.7	826.1	537.0	2926.0
12	15.2	92.0	307.9	871.2	617.8	3029.7
13	17.7	95.5	339.9	913.7	737.9	3096.7
14	18.9	98.1	365.0	1146.7	882.1	4184.6
15	19.8	103.1	451.2	1461.8	1017.6	4473.3
16	20.2	106.4	538.5	1622.7	1171.0	4538.4
17	20.3	111.0	604.9	1724.8	1282.0	4666.5
18	—	—	—	—	—	—
19	—	—	—	—	—	—
20	—	—	—	—	—	—

trial ranged from 2.44 to 17.84%, the latter value being yielded by the sample at 25.2% moisture. All the other interseed carbon dioxide values were below 12% up to the final day of the trial. Subsequent studies indicated that marked inhibition of respiration is to be ex-

pected at interseed carbon dioxide concentrations in excess of 12%, so that the respiratory rate of the sample containing 25.2% moisture is doubtless an underestimation of the respiratory rate attainable under adequate aeration conditions.

In the sample at 38.6% moisture a considerable number of seeds germinated, some sprouts having a length up to 5 mm. This germination probably occurred during the first few days of the trial and ceased with the death of the seedling from the toxic effects of increasing mold growth. No seeds germinated in any of the other lots.

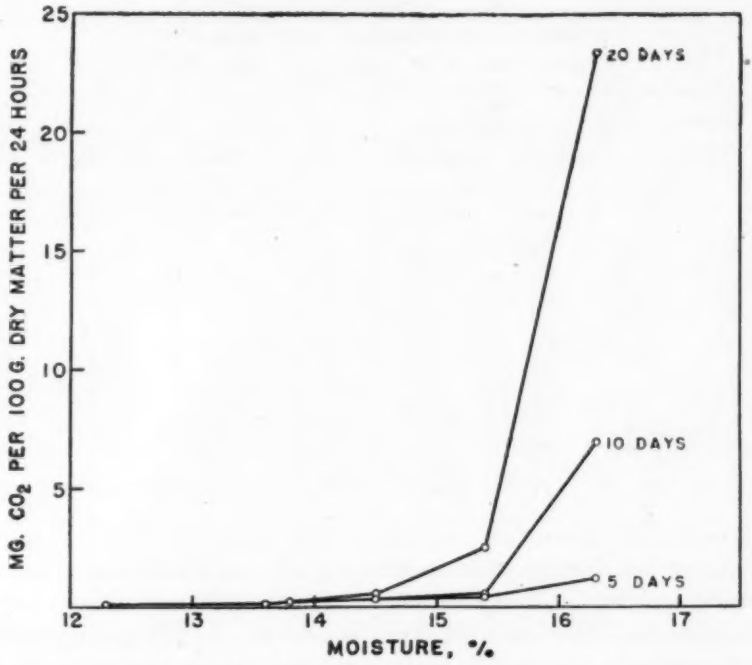

Fig. 1. Influence of moisture content and time on the respiratory rate of Regent wheat at 30°C.

Table I shows that the respiratory activity of wheat at moisture values in equilibrium with relative humidity values below 74% was virtually constant over the entire period of the test (20 days). On the other hand, beginning with moisture contents of 14.5% (75% relative humidity), respiratory rates increased with time following an induction period the length of which is inversely proportional to the moisture content. This induction period probably represents the time required for mold spores to germinate and for mycelia to become established. In any event the number of mold colonies per gram at the end of the respiration trial appears to be correlated with the ultimate respiratory rates. The respiration-time curves for moisture

values at which mold growth occurs assume the form of a microbiological growth curve. Curves in Figure 1 relating respiratory activity to moisture content assume increasingly sharper inflections with time in the critical moisture range (14.5–15%), corresponding to a relative humidity of 74–75%.

During the test the moisture content of those samples originally at 18.5% moisture or more increased significantly, as is shown in Table II. Samples having initial moisture contents above 14.5% showed an

TABLE II

RELATIONSHIP OF MOISTURE CONTENT TO RESPIRATORY RATE, CHEMICAL COMPOSITION, AND VIABILITY
(Regent wheat after respiratory trials at 30°C)

Moisture content		Time in respirometers	Respiratory rate (final day)	Fat acidity	Total sugars	Reducing sugars as % of total	Germination
Initial	Final						
%	%	*Days*	*mg* CO_2	*mg KOH/10 g*	*mg/10 g*	%	%
12.3	12.0	20	0.07	35.3	252	19.0	93
13.6	13.1	20	0.11	35.5	263	17.9	95
13.8	13.7	20	0.23	35.3	237	19.8	95
14.5	14.3	20	0.57	37.8	252	19.8	92
15.4	14.6	20	2.53	42.3	255	20.0	91
16.3	16.0	20	23.35	66.0	248	21.8	67
16.8	16.4	17	20.3	38.6	247	20.6	88
18.5	19.0	17	111.0	115.0	225	21.8	37
20.8	22.0	17	604.9	149.7	202	27.2	14
25.2	30.0	17	1724.8	140.7	184	33.7	21
30.5	34.1	17	1282.0	155.5	171	46.2	—
38.6	48.1	17	4666.5	392.1	270	58.5	—

increase in fat acidity with respiratory rate, mold proliferation, and moisture content. Conversely, total sugars, while remaining virtually unchanged until the moisture contents exceeded the critical level, declined markedly thereafter with increasing moisture content. The sole exception to these trends was the sample initially at 38.6% moisture in which many seeds germinated. Here total sugars *increased* during the course of the trial in spite of the fact that respiratory activity reached the highest level of any sample. The reducing sugar fraction did not change significantly at moisture contents up to 15.4% but increased regularly thereafter with increasing moisture content. Seed germination was unaffected at moisture contents below 15.4%, but decreased sharply with increasing moisture values, coincident with increasing mold growth and respiration.

The relationship between respiratory quotient and moisture content shown in Table III appears to be a complex one. At moisture values below that necessary for molds to grow, that is from 12.3 to

TABLE III

INFLUENCE OF TIME AND MOISTURE CONTENT ON THE RESPIRATORY QUOTIENT OF REGENT WHEAT

Day	Respiratory Quotient					
	Moisture content, %					
	12.3	13.6	13.8	14.5	15.4	16.3
3	0.57	0.77	0.76	0.87	0.91	0.96
5	0.53	0.66	0.81	0.89	0.90	0.96
7	0.37	0.59	0.83	0.81	0.85	0.92
9	0.61	0.70	0.76	0.88	0.92	0.86
11	0.57	0.62	0.76	0.82	0.85	1.01
13	0.50	0.74	0.83	0.94	0.79	0.96
15	0.47	0.72	1.00	0.94	0.78	0.92
17	0.60	0.85	0.91	0.89	0.78	0.91
19	—	—	—	0.92	0.78	0.90
Day	Moisture content, %					
	16.8	18.5	20.8	25.2	30.5	38.6
3		1.17	1.04	0.96	0.96	0.99
5	1.91	1.10	0.95	0.93	0.97	1.04
7	1.38	0.99	0.87	0.91	0.95	1.05
9	1.08	0.92	0.85	0.93	0.95	1.03
11	1.06	0.88	0.86	0.97	0.97	1.01
13	1.01	1.06	0.88	0.98	—	0.98
15	0.95	0.84	0.93	0.97	1.00	0.97
17	0.90	0.81	0.93	0.99	1.00	0.97
19	—	—	—	—	—	—

13.8% moisture, the respiratory quotient increased from about 0.5 to 0.8 with increasing moisture, and there was no significant change in the respiratory quotient with time. At 14.5% moisture, in the critical range for the initiation of mold growth, the respiratory quotient remained essentially at 0.9 throughout the trial. On the other hand, at 15.4% moisture, where molds became well established, a value of 0.9 was maintained only for the first 9 days, followed by a drop in the respiratory quotient to 0.8 in the later days of the trial. This decrease of respiratory quotient coincident with increasing proliferation and diversity of mold population was most apparent in the central range of moisture values between 15 and 19%. At moisture levels approaching the germination requirement, the respiratory quotient approached a common level of 1.0.

Relation of Moisture Content to Mold Growth

The effect of the conditions of the respiration trials on mold growth is shown in Table IV. The seed used was relatively free of mold con-

tamination. Approximately 65% of the seeds were internally infected with *Alternaria*, and less than 5% by *Helminthosporium*. No other internal molds were found. Neither of these fungi is able to grow in seed with a moisture content below 25%, and, indeed, they are soon eliminated from seed stored at moisture contents above 16%, apparently because they are unable to compete with the more aggressive molds such as *Aspergillus* and *Penicillium*.

TABLE IV

RELATIONSHIP OF MOISTURE CONTENT TO RESPIRATORY RATE AND MOLD GROWTH

(Regent wheat after respiratory trials at 30°C)

Moisture content, initial	Respiratory rate (final day)	Mold colonies per g	*A. glaucus*	*A. flavus*	*A. candidus*	*A. ochraceus*	*A. niger*	*Penicillium* sp.	*Rhizopus*
%	mg CO_2		%	%	%	%	%	%	%
12.3	0.07	500	*Penicillium* and *Aspergillus*						
13.6	0.11	100	*Penicillium* and *Aspergillus*						
13.8	0.23	100	*Penicillium* and *Aspergillus*						
14.5	0.57	400	*Penicillium* and *Aspergillus*						
15.4	2.53	4,800	All *A. glaucus*						
16.3	23.35	396,000	Mostly *A. glaucus*, a few penicillia						
16.8	20.3	209,000	95.7	2.2	1.2	—	—	0.9	—
18.5	111.0	2,275,000	16.0	2.1	74.9	0.6	—	6.4	—
20.8	604.9	11,300,000	1.8	0.9	78.8	1.3	—	17.2	—
25.2	1724.8	37,500,000	—	56.0	14.7	—	—	29.3	—
30.5	1282.0	63,500,000	—	59.1	—	—	6.3	34.6	—
38.6	4666.5	67,000,000	—	76.9	—	—	2.2	15.7	5.2

Some mycelium was visible in the inner side of the pericarp of all of the seeds examined, being more common near the brush and germ ends. Oxley and Jones (1944), who first described a mycelium within the pericarp of wheat seed, suggested that this mycelium was mainly responsible for the respiration of moist seeds. They did not determine whether the mycelium they observed was capable of growing at the moisture contents they used, or even if it was alive. In the present studies strips of pericarp from 15 surface-disinfected seeds yielded a few colonies of bacteria, and several cultures of *Alternaria*. No *Aspergillus* or *Penicillium* were recovered from the inner pericarp of this or two other lots of wheat similarly cultured. The surface disinfection may have killed some of this mycelium, but it is well known that the mycelium of *Aspergillus* and *Penicillium* is relatively short lived when subjected to desiccation, while the spores may remain alive for years in a dry state. Nonsurface-disinfected seeds from samples on which respiration trials were carried out, which had originally from 100 to 500 molds per gram, all of them being *Aspergillus* and *Penicillium*,

were ground in a Wiley mill as previously described. It seems likely that the mold flora which developed on the samples in these respiration tests came largely from inoculum on the outside of the seed, probably in the form of spores.

At moisture contents of 12.3 to 14.5% the mold count did not increase during the period of the test. *Aspergillus glaucus* began to develop at a moisture content of 14.5%, and grew vigorously at 15.4 and 16.3% moisture. *A. glaucus* is among the most xerophytic of the molds; and Snow, Crichton, and Wright (1944) have shown that some strains of this species will grow even at relative humidities below 75%. As the moisture content was increased above 16.8%, *A. glaucus* fell off rapidly; *A. candidus* made up more than 75% of the mold flora in the samples at 18.5 and 20.8% moisture; and at the higher moisture contents *A. flavus* comprised more than 50% of the mold flora, with *Penicillium* increasing in the range of 25.2 to 30.5% moisture.

Work with other samples of wheat from various sources indicated that the types of molds associated with respiration at the moisture contents used in the present tests are generally present on all seeds. A sample of wheat from the irrigated region of Montana, in which no mycelium could be found under the pericarp, was conditioned to various moisture contents and stored in bottles. At 16% moisture the seed was overgrown with *A. glaucus;* at 17% *A. candidus* became the principal mold; and above 20% *A. flavus* became dominant. The picture was essentially the same as that described in the present tests. Similar results have been obtained with other wheats. These molds may be considered part of the "normal" microflora of wheat seeds. They differ in their humidity requirements, and the moisture content of the seed is the critical factor which determines which one, or combination, will predominate.

Influence of Aeration and Interseed Carbon Dioxide Concentration on Wheat Respiration

To determine the effect of carbon dioxide concentration on the respiration of wheat, subsamples of a lot of Regent wheat conditioned to 20.4% moisture were aerated at five different rates for 9 days at 30°C. The sixth subsample received nitrogen containing 1.01% oxygen. The data obtained in this trial are given in Table V.

Respiration is somewhat inhibited at carbon dioxide concentrations in excess of about 7%, but inhibition does not become marked until the interseed carbon dioxide exceeds 12%. This result agrees approximately with the interseed carbon dioxide concentration which was found to be inhibitory to soybean respiration under similar conditions by Milner and Geddes (1945).

TABLE V

INFLUENCE OF AERATION RATE ON INTERSEED CARBON DIOXIDE CONCENTRATION, RESPIRATORY RATE, RESPIRATORY QUOTIENT, FINAL MOISTURE CONTENT, FAT ACIDITY, AND GERMINATION OF REGENT WHEAT

Aeration rate	Interseed CO_2 concentration			Respiratory rate		
	3rd day	5th day	9th day	3rd day	5th day	9th day
ml/g d.m./day	%	%	%	*mg* CO_2	*per 100 g d.m.*	*per 24 hours*
Original sample	—	—	—	—	—	—
0.16[1] (N_2)	5.60	8.99	12.41	6.4	10.1	13.0
3.2	7.28	15.47	18.69	40.3	85.3	101.1
6.4	4.78	11.47	16.14	52.8	126.2	178.2
12.5	3.02	6.67	12.44	65.2	143.3	269.3
18.9	2.02	4.39	8.86	65.6	142.7	289.7
25.1	1.64	3.49	7.32	70.5	150.2	316.5

Aeration rate	Respiratory quotient			Moisture		Fat acidity	Germination
	3rd day	5th day	9th day	Initial	Final		
ml/g d.m./day				%	%	*mg* KOH/*100 g*	%
Original sample	—	—	—	12.4	12.4	12.6	98
0.16[1] (N_2)	6.15	9.77	13.20	20.4	20.5	12.7	95
3.2	0.95	0.95	0.90	20.4	20.5	65.9	24
6.4	1.01	0.95	0.83	20.4	20.8	69.6	18
12.5	1.04	0.93	0.80	20.4	20.9	69.6	18
18.9	1.03	0.92	0.80	20.4	21.0	71.6	19
25.1	1.07	0.92	0.82	20.4	21.1	74.4	16

[1] Volume of air (20.93% oxygen) equivalent to nitrogen containing 1.01% oxygen.

The data for respiratory quotient show that with damp wheat respiring in air, where the major portion of the respiration can be attributed to mold growth, the respiratory quotient decreased with time, from an initial value of approximately 1.0 to about 0.8 as the respiration trial progressed. This behavior was a general characteristic of damp wheat respiration in this moisture range. At the lowest aeration rate (3.2 ml per g dry matter per day) where the interseed carbon dioxide concentration reached a value of 18.7% and carbon dioxide production was markedly suppressed in comparison to that at higher aeration rates, no tendency toward an anaerobic type of respiration appears as indicated by the respiratory quotient. Actually the respiratory quotient remained well below 1.0 throughout the period of the trial. Only under an atmosphere of nitrogen containing 1.01% oxygen (equivalent to an aeration rate of 0.16 ml air per g dry matter per day) did the respiratory quotient assume a strongly anaerobic character (6.15 to 13.20).

The data again show that increasing respiratory rates indicative of a growing mold population are accompanied by increases in moisture

content of the grain samples, in spite of the controlled humidity of the seed atmosphere. Increased fat acidity and loss of germination accompany mold growth. On the other hand, where mold growth was virtually inhibited (nitrogen atmosphere), fat acidity and germination remained essentially constant, in spite of the elevated moisture value used in this trial. It may be concluded that under normal conditions of storage at high moisture contents, the lipase activity of wheat is insignificant in comparison to that of the molds.

Adiabatic Heating and Respiration of Damp Wheat

Using the adiabatically controlled thermostats and other experimental techniques described by Milner and Geddes (1946a), the heating and respiration of wheat at moisture contents of 18 and 22% were investigated.

Graphical data for a heating study on Regent wheat at 18% moisture appear in Figure 2. The curves are similar to those reported for soybeans by Milner and Geddes (1946). Following an initial induction period corresponding to the time required for mold spore germination and establishment of mycelium, increases in temperature and respiratory rate proceed in an exponential manner until the mold thermal death range (52°–55°C) is reached. This destruction of the major respiratory and thermogenic agents, i.e., the molds, resulted in a rapid fall in respiratory rate and a corresponding cessation of heating. On the 17th day of the trial, the thermostat temperature was artificially raised to 63.7°C, but no renewed heating appeared. Instead, a slight cooling, characteristic of the instrument calibration at this temperature, occurred. The secondary heating phase which was obtained regularly with soybeans, as previously reported, following thermal destruction of the mold population, does not occur readily with wheat, as it appears to require more strict control of adiabatic conditions than could be obtained in this trial. That such nonbiological heating can occur in wheat under more strictly controlled conditions will be reported in a later portion of this paper.

The initial course of the respiratory quotient in this heating experiment is analagous to the data obtained for damp wheat respiring at constant temperature. In the initial stages the respiratory quotient is approximately 1.0 and then falls slowly to a value of 0.9 in the range of increasing respiratory and thermogenic activity due to a proliferating mold population.

When the mold and seed thermal death range is reached, as marked by a reversal in carbon dioxide production and cessation of heating, the respiratory quotient rises sharply to a value in excess of 2 in two days and then decreases as sharply to 0.5 to 0.6. These characteristics are

exactly similar to those reported for soybeans with the exception that the low point in the respiratory quotient for the latter, in the region following the thermal destruction of seed and mold viability, was between 0.4 and 0.5.

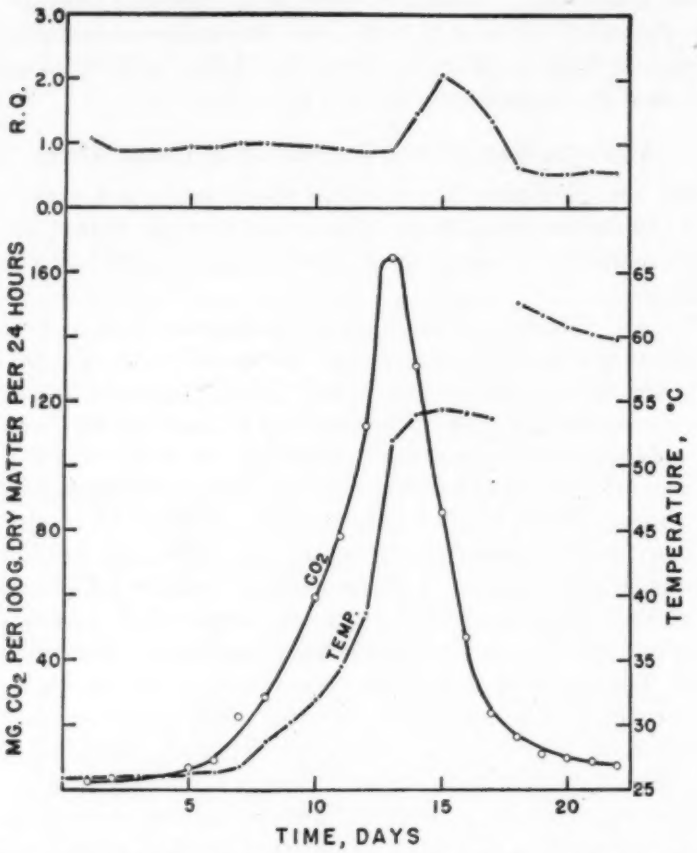

Fig. 2. The course of adiabatic heating and respiratory characteristics exhibited by Regent wheat at 18% moisture. The temperature was artificially raised to 63.7°C on the 17th day.

Data obtained on the wheat samples at various intervals in the heating trials (see Table VI) showed a progressive decrease in total sugars, a steady rise in reducing sugars, decreased germination, and increased mold growth up to the mold thermal death range. Fat acidity increased steadily in the range of mold proliferation but decreased at more elevated temperatures, in and beyond the mold thermal death range.

Using an electronic potentiometer device which allows for precise adjustment and control of adiabatic conditions, a heating study was conducted with Regent wheat containing initially 22% moisture (equi-

TABLE VI

RELATION OF ADIABATIC HEATING TO CHEMICAL DETERIORATION, GERMINATION, AND MOLD PROLIFERATION

Temperature at time of sampling	Day	Fat acidity	Total sugars	Reducing sugars as % total	Germination	Mold infected seeds	Mold colonies per seed
		mg KOH/*100 g*	*mg*/*10 g*	%	%	%	
—	0	34.5	252	18.3	94	—	5
26.4	6	44.9	252	18.3	—	60	—
28.8	8	52.8	243	18.5	87	95	5,600
32.0	10	73.3	249	19.7	78	85	33,000
52.0	13	72.9	228	28.1	23	23	20,600
54.1	17	71.0	199	49.7	0	0	0
60.7	20	62.3	205	59.0	0	0	0
59.8	22	43.6	200	65.0	0	0	0

librium relative humidity 94%) for 46 days. The data from this experiment are shown in Figure 3 in the form of curves relating respiratory rate, temperature, daily temperature change, and respiratory quotient to time.

In the heating range due to mold respiration, the results are similar to those in the previous trial. In addition, however, following the expected reduction in respiration and heating after thermal destruction of the molds, a slowly increasing respiratory rate (14th day), and eventually a new heating (17th day), appeared, which reached a maximum of carbon dioxide production on the 27th day and a temperature maximum (68°C) on the 32nd day. The temperature maximum was achieved in spite of a calibrated cooling rate of about 1°C per day in this temperature range. Respiratory and thermogenic inhibition appeared at this time in a manner similar to that which involved mold heating and respiration at a lower temperature (50°–55°C). Although this sample was not examined for microflora, these data suggest that thermophilic bacteria were responsible for this secondary heating phase. The initial moisture content of the sample (22%) was close to the hygroscopic moisture equilibrium (95% relative humidity) where bacterial proliferation is to be expected.

On the 36th day the control instrument was adjusted to make temperature conditions more nearly adiabatic. Heating of the sample commenced again at once, and the respiratory rate showed a new upward trend. Between the 37th and 45th day, when the trial was discontinued at 93.8°C, the heating and carbon dioxide evolution resembled closely the characteristics of soybean heating in a similar range due to nonbiological oxidation (Milner and Geddes, 1946).

On completion of the trial, the adiabatic calibration of the thermostat control was checked at various temperatures with water in the Dewar flask. The results of this check as well as the calibration settings used in the trial appear in Table VII.

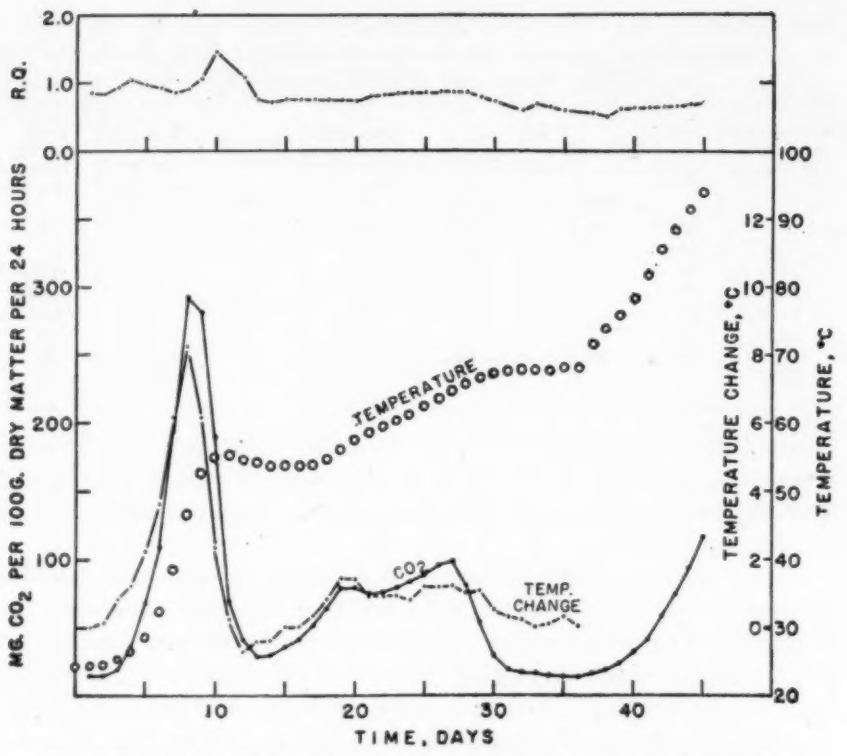

Fig. 3. Respiratory and heating characteristics of Regent wheat under strictly controlled adiabatic conditions at a moisture content of 22%.

These data show conclusively that the heating trends obtained in the experiment were due to thermogenic processes in the grain sample which took place in spite of the fact that it was impossible to maintain uniform adiabatic conditions over the entire temperature range. The data for the last several days of the trial are particularly significant since they show that in the neighborhood of 90°C, where heating of the seeds was progressing very rapidly, the cooling rate of the system was 2.7°C per day.

TABLE VII

TEMPERATURE CHANGES OF ADIABATIC THERMOSTAT AT VARIOUS TEMPERATURE RANGES AND CALIBRATION VALUES

Temp. range of trial	Calibration value of instrument	Initial checking temperature	Temp. change in 24 hours
°C	*Units*	°C	°C
24.2–65.6	78	33.3	−0.4
24.2–65.6	78	59.7	−1.7
65.6–68.0	90	62.6	−0.8
68.0–93.8	110	75.6	0.0
	110	90.0	−2.7

Discussion

The results of this study dealing with the characteristics of wheat respiration and deterioration supplement the previous results of Milner and Geddes with soybeans, and lead to the conclusion that the basic phenomena involved in the deterioration of both seed types are essentially similar. It is clear that at least for the cereal grains, and for soybeans and flaxseed, the critical moisture levels (where sharp respiratory increases and chemical deterioration commence) coincide with the minimal atmospheric humidity values at which mold proliferation is initiated. For sound seeds this critical relative humidity is 74–75% at normal temperatures.

The respiration of wheat seed at moisture contents in equilibrium with relative humidities below 74% remains constant for at least 20 days at constant temperature (Table I and Figure 1). On the other hand, at humidities in excess of 74%, mold growth commences and is accompanied by a continuously increasing respiratory rate. The lag period of mold spore germination decreases with increasing moisture content and humidity. The progressively higher initial respiratory rates before the mold growth begins, as well as the uniform constancy of the respiratory rates at humidities where mold growth is not involved (below 74%), suggest that these respiratory rates are due to true seed respiration.

The respiratory quotient of damp wheat increases with moisture content from values of about 0.6 at 12.3%, where mold growth is not involved, to values in the range of 0.8 to 1.0 at moisture contents where molds are the principal contributors to the respiration. These facts suggest that the assumption which has been made that wheat respiration is characterized by respiratory quotients of unity regardless of moisture value is untenable. The corollary assumption that carbohydrates are the principal materials respired, which appears to be verified by the strong decrease in total sugars as a result of respiration (Table II), is similarly not reflected in the data for respiratory quotient. The unity value for respiratory quotient appeared only in the initial phases of mold growth and at moisture values close to the seed germination threshold. Any interpretation of the significance of respiratory quotient in respiring seeds must take into account not only the data presented here, which show that different species of molds predominate at different moisture levels, but also the possibility that the gas exchange in seeds may be affected by physical and chemical factors other than those involved strictly in substrate utilization.

The change in predominance of various mold types with increasing moisture content (Table II) indicates the mixed nature of microfloral infection in wheat and suggests that an interspecies competition exists

which is primarily dependent on the optimum moisture requirement of different mold types. Thus, the extremely xerophytic *Aspergillus glaucus* predominates on samples up to 16.8% moisture (83% relative humidity), while at higher moisture levels a preponderance of *A. candidus* appears, along with Penicillia to a lesser extent. *A. flavus* becomes the major organism at moisture values in excess of 25%; Penicillia also increase in this range.

The regular decrease in total sugars, with increasing moisture content, which was shown to be related to respiratory activity (Table II), is to be expected since carbohydrates are usually the primary substrate utilized in the respiratory process. It is striking, however, that when seed germination occurred (38.6% moisture), a marked increase in total sugars appeared during the respiration trial, in spite of the extremely high respiratory activity. It may be inferred that certain enzymes, in this case probably wheat alpha-amylase, are activated only at high hydration levels, whereas others, such as the mold lipase, function at considerably lower moisture values.

Interpretation of the data for the influence of aeration on respiratory activity (Table V) must take into account the aeration process used in these studies. Since normal air is moved through a considerable mass of grain, low aeration rates will allow the seeds and molds in the immediate vicinity of the entering air stream to satisfy their oxygen requirements fully, whereas those at a greater distance will encounter progressively lower oxygen concentrations. Thus, at low aeration rates it might be postulated that a portion of the seed samples respire under relatively anaerobic conditions; and that progressively decreasing aeration should be reflected in elevated respiratory quotients, since the respiratory quotient values would be an average of the respiratory characteristics in the wide range of oxygen concentration existing. However, this does not appear to be the case, since the respiratory quotients do not increase progressively with increasing respiratory suppression as a result of low aeration rates (Table V). Only under nitrogen which contained 1.01% oxygen (equivalent to 0.16 ml air per g dry weight per day) did respiratory quotients indicating an anaerobic tendency appear.

The data suggest, therefore, that the principal effect of limited aeration was to reduce the extent of mold growth and that the lowest aeration supplied (3.2 ml per g dry weight per day) was sufficient to maintain a normal aerobic type of respiration both of the seed and of the small mold population which was able to grow. However, under nitrogen (1.01% oxygen) the respiratory quotient values reflect a definite blocking of the aerobic process, which suggests that the critical range between aerobic and anaerobic respiratory characteristics was

intermediate between the two lowest aeration values used. That seed, rather than mold, respiration was the principal process involved under the condition of lowest oxygen supply (nitrogen with 1.01% oxygen) seems probable because of the negligible increases in respiratory rate with time, as well as the insignificant changes in the values for fat acidity and seed germination over the course of the trial.

The characteristics of the heating and respiration of wheat under adiabatic control are similar to those for soybeans reported by Milner and Geddes (1946). It seems quite clear that the initiation of heating of any biological material capable of sustaining microbiological growth is due to mold proliferation, which ceases at the mold thermal death range of 50°–55°C. At a relative humidity of 95% or more, bacterial growth and respiration may cause temperature increases to 68° to 70°C. These temperature limits for microbiological growth on agricultural materials have been well established by many workers, as reviewed in a previous paper in this series (Milner and Geddes, 1946a).

Under strictly controlled adiabatic conditions, spontaneous nonbiological oxidation appears beyond the bacterial death range, even with wheat. Soybeans, however, showed this secondary heating more readily than did wheat, owing probably to their higher content of unsaturated triglycerides.

Certain similarities and minor differences exist in the respiratory quotient of wheat in comparison to soybeans. In the temperature range in which mold growth occurs, the respiratory quotient of wheat showed a decrease with time from about 1.0 to 0.9. Soybean respiration showed somewhat lower initial respiratory quotient values, which tended to reach a value of 1.0 as the thermal death range was approached. Both seed types, however, exhibit the sharp rise in respiratory quotient coincident with the thermal killing of the seeds and molds. Milner and Geddes (1946a) showed that this effect was due to the seeds and not to the dying mold population since sterilized soybeans which were reinoculated with a mold culture failed to show this rise. Following the thermal death of seeds and molds, the respiratory quotient in wheat was about 0.75, whereas soybeans under similar conditions showed a respiratory quotient of 0.45. An increase in respiratory quotient to about 0.9 accompanied what was probably bacterial proliferation, but this trend also is reversed when the thermal death range of these microflora (68°–70°C) is reached, the values then dropping to about 0.6. When purely nonbiological oxidation in wheat was in progress, the respiratory quotient rose regularly, in a manner very similar to the gas exchange of soybeans heating spontaneously in a similar temperature range.

The decrease in fat acidity of wheat seeds at temperatures above the mold thermal death range was a striking feature in these studies. Further investigations of this phenomenon need to be made.

The close parallelism between respiratory rate and rate of temperature increase shown in Figure 3 is of interest since it has been suggested by Sallans *et al.* (1944) that such parallelism is not to be expected under conditions of restricted aeration. Other data from these experiments do not appear to verify this contention. As shown in Table IV, the aeration rate of samples exceeding from 14.5 to 15.0% moisture directly influences the extent of mold proliferation and consequent respiration and heat production, but not the characteristics of the respiration (as indicated by a change in respiratory quotient). This point has already been discussed, but it seems appropriate to conclude that molds, being aerobic, would be limited in growth by low aeration or low oxygen tension, and it is the extent of this growth which will be directly correlated to the degree of heating at moisture contents commonly encountered in stored grain.

Summary

The influence of moisture content and aeration on the respiratory characteristics of Regent wheat at 30°C was investigated by a technique providing for continuous aeration of samples and daily measurement of oxygen consumption and carbon dioxide production over prolonged time intervals. Adiabatic heating of damp wheat, as well as the role of molds in the respiratory, heating, and deteriorative processes, was studied.

Moisture contents in wheat below about 14.5% (corresponding to a relative humidity of 74–75%) yielded low and constant respiratory rates over extended time intervals, indicative of purely seed respiration. No significant increases in mold population, or chemical and germinative deterioration of the seeds, appeared in this low-moisture region. At moisture values beyond this critical humidity zone, respiratory rates increased with time following induction periods inversely proportional to the moisture content. Respiratory increases, which assumed the form of microbiological growth curves with time, were accompanied by mold growth, chemical deterioration of the seeds, as indicated by increases in fat acidity and reducing sugars, loss of germination, and increases in the moisture content of the wheat samples.

Respiratory quotient values increased at low moisture values below the critical moisture (14.5%) from 0.6 to 0.8, showing no material change with time. At more elevated moisture values initial respiratory quotients at or near unity were followed by regular decreases to

about 0.8 with time and increasing mold growth. Wheat samples with moisture contents at or near the germination requirement exhibited respiratory quotient values of unity over prolonged time intervals.

The mold species *Alternaria* and to a lesser extent *Helminthosporium* were the principal flora infecting sound, dry wheat seeds internally. At moisture values above the critical level, however, the predominant species appeared in the following order with increasing moisture content: *Aspergillus glaucus*, *A. candidus*, *A. flavus*, *Penicillium* sp. The extent of mold proliferation with time was directly related to the respiratory activity and extent of chemical and germinative deterioration of wheat samples.

Aeration rates yielding interseed carbon dioxide concentrations in excess of 7% were slightly inhibitory to the respiration of damp wheat, while marked respiratory inhibition appeared at carbon dioxide concentrations in excess of 12%.

Temperature and respiratory increases in damp wheat samples maintained under adiabatic conditions were directly correlated to mold growth until a temperature range of 52°–55°C is attained where molds are killed, respiration is inhibited, and heating ceases. At more elevated moisture values (95% humidity) bacterial growth may cause wheat to heat to the bacterial thermal death range of 68°–70°C. Under strictly controlled adiabatic conditions wheat may continue to heat spontaneously owing to nonbiological oxidation.

Literature Cited

American Association of Cereal Chemists
1941 Cereal Laboratory Methods (4th Ed.) American Association of Cereal Chemists, Lincoln, Neb.

Christensen, C. M.
1946 The quantitative determination of molds in flour. Cereal Chem. **23**: 322–329.

Milner, M., and Geddes, W. F.
1945 Grain storage studies. II. The effect of aeration, temperature, and time on the respiration of soybeans containing excessive moisture. Cereal Chem. **22**: 484–501.
1946 Grain storage studies. III. The relation between moisture content, mold growth, and respiration of soybeans. Cereal Chem. **23**: 225–247.
1946a Grain storage studies. IV. Biological and chemical factors involved in the spontaneous heating of soybeans. Cereal Chem. **23**: 449–470.

Oxley, T. A., and Jones, J. D.
1944 Apparent respiration of wheat grains and its relation to a fungal mycelium beneath the epidermis. Nature **154**: 826–827.

Sallans, H. R., Sinclair, G. D., and Larmour, R. K.
1944 The spontaneous heating of flaxseed and sunflower seed stored under adiabatic conditions. Can. J. Research **F22**: 181–190.

Snow, D., Crichton, M. H. G., and Wright, N. C.
1944 Mould deterioration of feeding-stuffs in relation to humidity of storage. Part I. The growth of moulds at low humidities. Ann. Applied Biol. **31**: 102–110.

A STUDY OF SOME PASTE CHARACTERISTICS OF STARCHES WITH THE CORN INDUSTRIES VISCOMETER

W. G. BECHTEL[1]

Corn Industries Research Foundation, New York, New York

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During recent years there has been increasing interest in methods for the continuous determination of the apparent viscosity of starch pastes. Several instruments have been described following the work of Caesar (1932). Among these are the Bauer viscometer described by Glabe (1939), which like Caesar's is designed for very concentrated pastes, the viscometer of Barham, Wagoner, and Reed (1942), and the Brabender Amylograph, which was used for studies of wheat starch by Anker and Geddes (1944).

More recently the Corn Industries Viscometer was developed for use as a standard testing instrument for the corn starch industry (Fig. 1). It has been described by Kesler and Bechtel (1947). In this instrument viscosity is determined and recorded continuously for a cooking period of any desired length, and during cooling of the paste also, if this is required. Variations in results due to differences in technique of preparing the paste are eliminated through thermostatic control of water bath temperature and mechanical stirring at constant rate. Evaporation of moisture is prevented by use of an efficient condenser built into the cover for the starch pasting beaker. A stirring device of unique design serves to remove the layer of pasted starch from the walls of the beaker, which greatly improves the efficiency of heat transfer to the body of the paste and results in a nearly uniform temperature of the contents. A separately mounted propeller driven through a gear differential stirs the center of the paste and serves as a means of measuring the viscosity changes. The stirrer produces smooth pastes, entirely free from lumps, without the necessity of any auxiliary hand stirring. To measure viscosity, the force which the propeller encounters is continuously balanced by a dynamometer consisting of a weight arm which moves through a vertical arc. Attached to the dynamometer is the pen of the strip type recorder. In order to attain equal sensitivity with dilute pastes or those of thin boiling starches as with concentrated pastes or thick boiling starches, readily interchangeable weights are provided for the dynamometer so that it covers torque ranges of 250, 500, 1,000, and 2,000 gram-centimeters.

[1] Address: C.I.R.F. Research Fellow ,Penick and Ford, Ltd., Inc., Cedar Rapids, Iowa.

Over an extensive testing period it has been found that duplicate determinations agree within plus or minus 1%.

The kind of chart record obtained is shown in Figure 2. In other figures the curves have been regraphed in rectangular coordinates for convenience in comparing related curves. Data presented in the tables have been taken from similar curves to summarize the essential information they give.

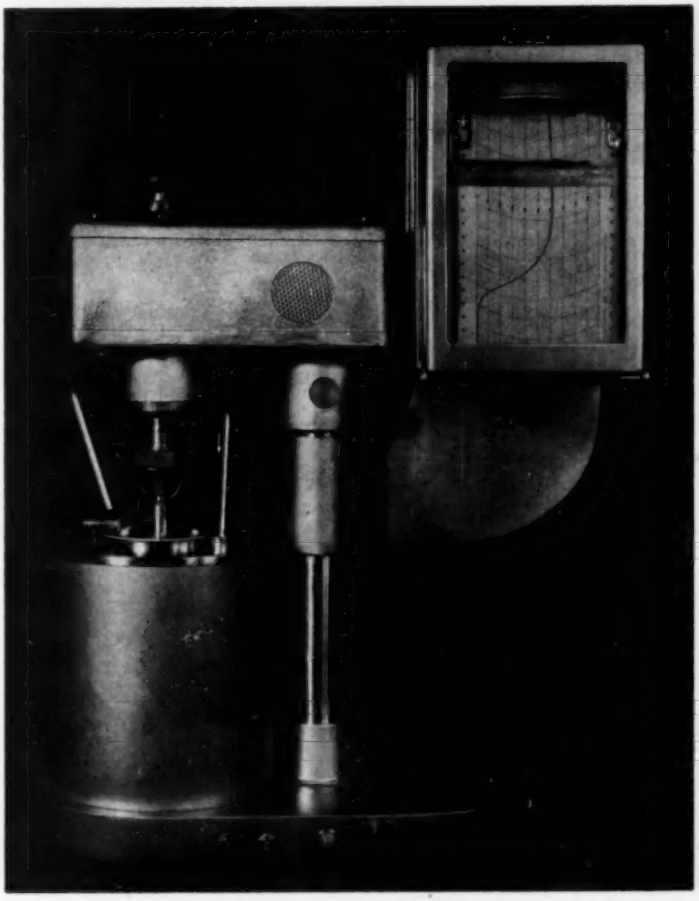

Fig. 1. Corn Industries Viscometer.

A technique of operation has been developed by which a viscosity curve sufficient for most industrial purposes may be obtained in 20 minutes or less, depending on the starch tested. In this method the water bath of the viscometer is preheated to the desired temperature (usually between 92° and 96°C), the motor-driven stirrer is started, and the starch-water slurry made with water at room temperature is

added. At this time the recorder is started and the condenser cover is put in place. The rest of the test is entirely automatic.

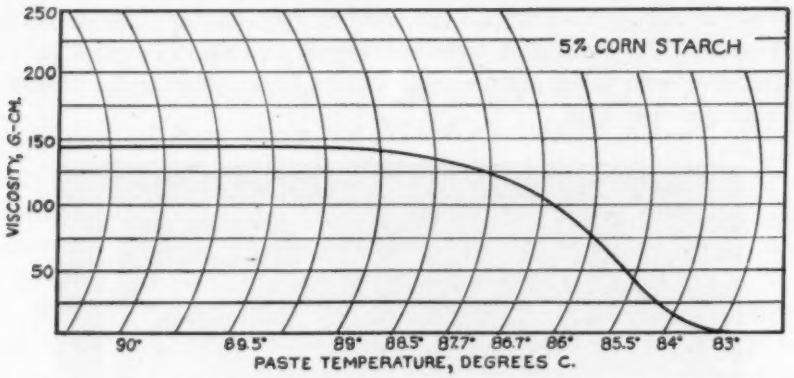

Fig. 2. Corn Industries Viscometer chart for 5% unmodified corn starch. Chart reads from right to left. Arcs are at 1-minute intervals.

The necessity for a continuous record of starch viscosity instead of a single test after a definite period of cooking, if a starch is to be characterized with certainty, is shown in Figure 3. It will be seen

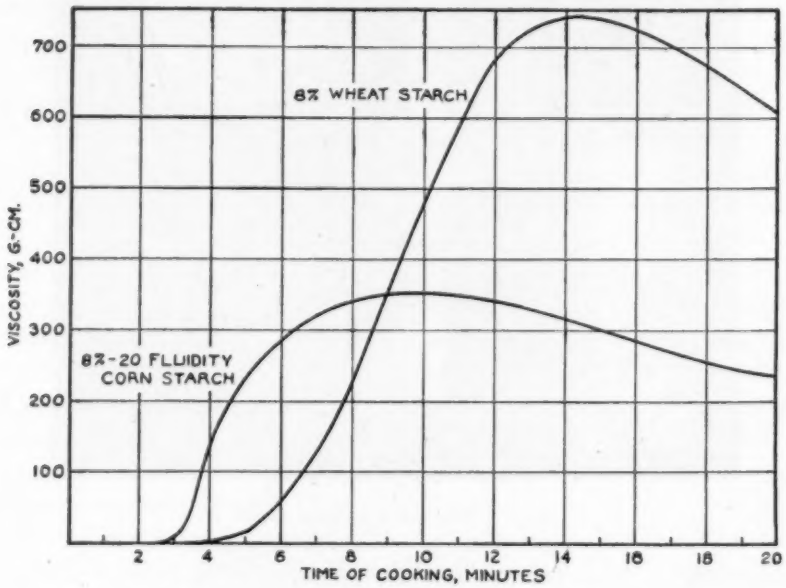

Fig. 3. Curves for wheat and an acid-modified corn starch.

that if a single viscosity test is made with an orifice or rotational viscometer under the paste conditions which exist at about 9 minutes' cooking in the Corn Industries Viscometer, the two starches will appear

to be nearly identical. Under the conditions at 15 minutes, where wheat starch reaches maximum viscosity, their differences are somewhat exaggerated. When single tests are made after a definite time of cooking, various starches may be tested at entirely different parts of the viscosity curve, depending on the gelatinization ranges and rates of the starches being studied. Such tests do not give a true comparison of paste properties. Such a comparison can only be made by a consideration of complete viscosity curves.

The present study was made to determine the effect of variations in rate of heating, final paste temperature, concentration of starch, and pH on the viscosity curves produced by the Corn Industries Viscometer, and to discover the effect of defatting on the viscosities of some corn starches. Throughout this work commercial starches were used, and it is recognized that other samples of the same designation may give somewhat different results if they were produced under different conditions of manufacture.

Effect of Rate of Heating

It has long been recognized that the rate of heating is one of the factors that must be controlled closely if results of duplicate tests made in an orifice viscometer are to agree closely. The reason for this is apparent, since such tests are made at the end of a definite time of cooking. From the literature examined, it has not been made clear whether the rate of heating the paste affects the viscosity of a starch when it is determined in a continuous reading viscometer.

Various rates of heating have been suggested for the several continuous reading viscometers. Caesar and Moore (1935) heated the paste at about 1°C per minute and the amylograph uses a rate of about 1.5°C per minute, starting at room temperature. Barham preheats the water bath to about 4°C below the gelatinization temperature of the starch being tested, then mixes the starch sample with water at this temperature and raises the temperature of the paste at 0.5°C per minute. The technique of Kesler and Bechtel has been given above.

Anker and Geddes (1944) studied the effect of the temperature of water used for the test. They reported that there was an increase in maximum viscosity when water above 45°C was used to prepare the paste. Caesar and Moore (1935) reported that at very rapid rates of heating the gelatinization temperature of tapioca starch was raised. They defined gelatinization temperature as the temperature of initial viscosity rise observed in the consistometer.

Two series of determinations were made. In the first, the water bath was preheated to 92°C, and the starch-water slurry was prepared at 25°C. In the second the water bath was at 25°C at the start and

the starch sample was prepared with water at the same temperature. The bath was then heated to 92°C at about 2°C per minute. Differences in rate of heating the paste are shown below.

Time, minutes	5	10	15	20	25	30	35	40
Paste temp. °C, bath 92°C	80°	88°	90°	90°	90°	90°	90°	90°
Paste temp. °C, bath 25°C, heated to 92°C	31°	42°	53°	64°	76°	85°	88°	90°

The viscosity curves obtained are shown in Figure 4, for 5% pastes of unmodified corn starch. The results indicate that rapid heating lowers the temperature of initial viscosity rise (sometimes called gelatinization temperature or temperature of transition). Rapid heating decreases the time required at 90°C for the starch to reach its maximum viscosity. With rapid heating the maximum was obtained 2 minutes after the paste reached 90°C, while with slow heating 7 minutes heating at 90° was required. Rapid heating increases the maximum viscosity to a marked extent and the difference in viscosity remains for the rest of the test. Similar results were found for samples from different manufacturers. This study is now being extended to include modified corn starches, starches from other natural sources, and other experimental conditions.

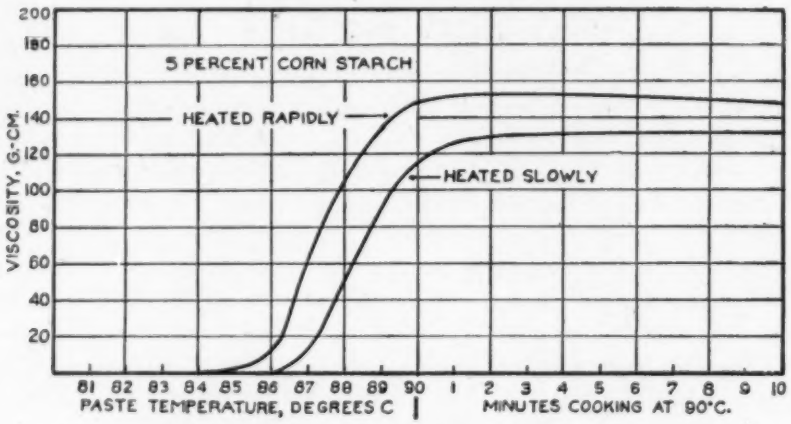

Fig. 4. Effect of a difference in rate of heating on the curves for corn starches.

Effect of Final Paste Temperature

Since the thermostat of the Corn Industries Viscometer is readily adjustable to any desired temperature, it has been possible to study the effect of varying the final paste temperature on the viscosity curves of starches. Unmodified corn and wheat starches, acid-modified corn

starches of alkali fluidity 40 and 90, and a highly oxidized corn starch were tested. The term alkali fluidity refers to the test developed by Buel (1912) and widely used in the corn starch industry to denote various grades of acid-modified starches. Hereafter the term fluidity will refer to alkali fluidity. The greater the degree of modification of starch the higher is the fluidity.

The water bath was preheated to the temperature required to obtain the desired final paste temperature. At higher bath temperatures the rate of heating the paste was increased to a slight extent. It was determined that this small change in rate of heating did not have an observable effect on the viscosity curves. The effects shown in Table I are due to the differences in temperature to which the pastes were heated.

In all cases the decrease in viscosity, or paste breakdown, from the maximum to the value after a total of 20 minutes' cooking becomes greater with higher temperature. This is generally known. However, the important fact shown is the great change in paste breakdown that may be caused by a temperature difference as low as 2°C. This appears especially pronounced in the case of wheat starch at 92° and 94°C, and for the modified starches. It indicates that when starches are stirred while cooking, a difference of more than a few tenths degree in final paste temperature produces readily noticeable changes in paste properties.

There appears to be a temperature for each starch at which it gelatinizes reasonably rapidly and at which breakdown is slow. For unmodified corn starch this is about 90°C, for wheat starch 92°, for 40 fluidity corn starch 86°, for 90 fluidity acid-modified corn starch 75°, and for the oxidized corn starch 67°. This is the case at the concentrations examined. As shown later, paste breakdown depends also on concentration.

The characteristic viscosity curve is the resultant of two opposite processes, according to Katz (1938). The first part of the curve is due to granule swelling, which increases with rise in temperature and which causes the observed viscosity rise. As the granules become more highly swollen they are more easily ruptured mechanically. Thus at higher temperatures granule breakdown occurs with resulting decrease in viscosity. It seems likely that a part of the decrease in viscosity is due to granule shrinkage because of the dissolving out of soluble material, and to softening of the granules which makes them more readily deformable.

Since the rates at which these opposing processes proceed are not necessarily affected to the same degree by change of temperature, it

TABLE I

EFFECT OF FINAL TEMPERATURE ON THE PROPERTIES OF STARCH PASTES

Final paste temperature	Maximum viscosity	Viscosity after 20 minutes	Breakdown[1]
°C	G-Cm	G-Cm	G-Cm
5% UNMODIFIED CORN STARCH			
88°	[2]	124	—
90°	164	164	0
92°	168	156	12
94°	165	140	25
5% UNMODIFIED WHEAT STARCH			
88°	[2]	13	—
90°	48	48	0
92°	48	43	5
94°	59	18	41
8% ACID-MODIFIED CORN STARCH, 40 FLUIDITY			
86°	118	118	0
88°	117	99	18
90°	130	72	58
92°	123	25	98
94°	119	0	119
25% ACID-MODIFIED CORN STARCH, 90 FLUIDITY			
75°	150	145	5
77°	168	136	32
83°	214	116	98
88°	230	56	174
94°	226	32	194
20% OXIDIZED CORN STARCH			
67°	150	150	0
70°	164	147	17
78°	200	110	90
88°	190	6	184
94°	178	0	178

[1] The difference between maximum and 20-minute viscosities.
[2] Maximum viscosity was not reached in 20 minutes at this temperature.

is not surprising that a change in final paste temperature often causes a change in maximum viscosity.

Effect of Concentration

Four effects due to changes in concentration of the paste are evident, as shown in Table II. Similar results were obtained with other starches. With increasing concentration the initial viscosity rise is

observed at a lower temperature, the rate of viscosity rise increases, and the extent of breakdown following the maximum becomes greater. Successive equal increases in concentration cause increasingly large differences in viscosity. Thus the change in concentration from 5 to 6% unmodified corn starch causes an increase in maximum viscosity of 162 g-cm, while the change from 9 to 10% causes an increase of 485 g-cm. These effects are similar to those obtained by use of other instruments by Caesar and Moore (1935), Barham, Wagoner, and Reed (1942), and Anker and Geddes (1944).

TABLE II

EFFECT OF CONCENTRATION ON VISCOSITY
(Wheat starch heated to 92°C. Other starches to 90°)

Concentration	Temperature of initial viscosity rise	Temperature at maximum viscosity	Maximum viscosity	Viscosity after 20 minutes	Breakdown[1]
%	°C	°C	G-Cm	G-Cm	G-Cm
UNMODIFIED CORN STARCH					
5.0	82.5	90	166	163	3
6.0	78.5	89	328	314	14
7.0	73.5	88	562	511	51
8.0	71	86.5	895	787	108
9.0	70	85.5	1255	1045	210
10.0	70	84.5	1740	1373	367
ACID-MODIFIED CORN STARCH, 40 FLUIDITY					
6.0	74	88	36	27	9
7.0	72	86.5	92	60	32
8.0	71	86	167	106	61
9.0	70	84.5	276	174	102
10.0	69	81	491	240	251
11.0	67.5	78	745	330	415
12.0	67.5	76	1105	415	690
13.0	—	—	1517	556	961
UNMODIFIED WHEAT STARCH					
5.0	86.5	91	48	42	6
6.0	82.5	91	188	145	43
7.0	75	91	418	341	77
8.0	70	91	741	612	129
9.0	64	91	1205	915	290
10.0	62	91	1760	1445	315

[1] The difference between the maximum and 20 minute viscosities.

Anker and Geddes (1944) reported that the graph of the logarithm of maximum paste viscosity against the logarithm of starch concentration was linear for potato, corn, and wheat starches. Results obtained

TABLE I

EFFECT OF FINAL TEMPERATURE ON THE PROPERTIES OF STARCH PASTES

Final paste temperature	Maximum viscosity	Viscosity after 20 minutes	Breakdown[1]
°C	G-Cm	G-Cm	G-Cm
5% UNMODIFIED CORN STARCH			
88°	[2]	124	—
90°	164	164	0
92°	168	156	12
94°	165	140	25
5% UNMODIFIED WHEAT STARCH			
88°	[2]	13	—
90°	48	48	0
92°	48	43	5
94°	59	18	41
8% ACID-MODIFIED CORN STARCH, 40 FLUIDITY			
86°	118	118	0
88°	117	99	18
90°	130	72	58
92°	123	25	98
94°	119	0	119
25% ACID-MODIFIED CORN STARCH, 90 FLUIDITY			
75°	150	145	5
77°	168	136	32
83°	214	116	98
88°	230	56	174
94°	226	32	194
20% OXIDIZED CORN STARCH			
67°	150	150	0
70°	164	147	17
78°	200	110	90
88°	190	6	184
94°	178	0	178

[1] The difference between maximum and 20-minute viscosities.
[2] Maximum viscosity was not reached in 20 minutes at this temperature.

is not surprising that a change in final paste temperature often causes a change in maximum viscosity.

Effect of Concentration

Four effects due to changes in concentration of the paste are evident, as shown in Table II. Similar results were obtained with other starches. With increasing concentration the initial viscosity rise is

observed at a lower temperature, the rate of viscosity rise increases, and the extent of breakdown following the maximum becomes greater. Successive equal increases in concentration cause increasingly large differences in viscosity. Thus the change in concentration from 5 to 6% unmodified corn starch causes an increase in maximum viscosity of 162 g-cm, while the change from 9 to 10% causes an increase of 485 g-cm. These effects are similar to those obtained by use of other instruments by Caesar and Moore (1935), Barham, Wagoner, and Reed (1942), and Anker and Geddes (1944).

TABLE II

EFFECT OF CONCENTRATION ON VISCOSITY
(Wheat starch heated to 92°C. Other starches to 90°)

Concentration	Temperature of initial viscosity rise	Temperature at maximum viscosity	Maximum viscosity	Viscosity after 20 minutes	Breakdown[1]
%	°C	°C	G-Cm	G-Cm	G-Cm
UNMODIFIED CORN STARCH					
5.0	82.5	90	166	163	3
6.0	78.5	89	328	314	14
7.0	73.5	88	562	511	51
8.0	71	86.5	895	787	108
9.0	70	85.5	1255	1045	210
10.0	70	84.5	1740	1373	367
ACID-MODIFIED CORN STARCH, 40 FLUIDITY					
6.0	74	88	36	27	9
7.0	72	86.5	92	60	32
8.0	71	86	167	106	61
9.0	70	84.5	276	174	102
10.0	69	81	491	240	251
11.0	67.5	78	745	330	415
12.0	67.5	76	1105	415	690
13.0	—	—	1517	556	961
UNMODIFIED WHEAT STARCH					
5.0	86.5	91	48	42	6
6.0	82.5	91	188	145	43
7.0	75	91	418	341	77
8.0	70	91	741	612	129
9.0	64	91	1205	915	290
10.0	62	91	1760	1445	315

[1] The difference between the maximum and 20 minute viscosities.

Anker and Geddes (1944) reported that the graph of the logarithm of maximum paste viscosity against the logarithm of starch concentration was linear for potato, corn, and wheat starches. Results obtained

with the Corn Industries Viscometer confirm their findings for corn and wheat starches within the limits of concentration tested. In addition, tapioca and acid-modified corn starches were found to give similar results, as shown in Figure 5.

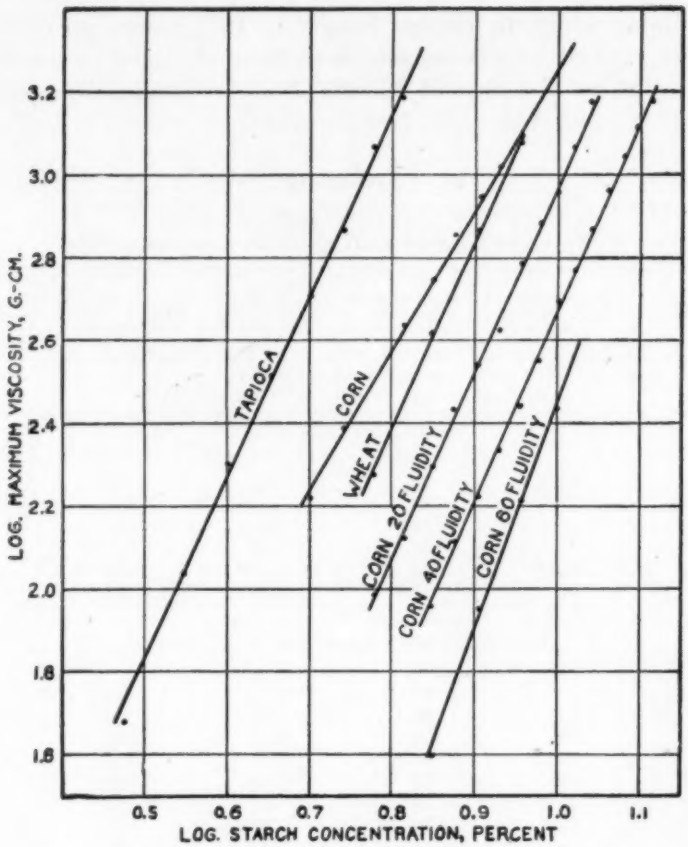

Fig. 5. Relationship between starch concentration and maximum viscosity.

Effect of pH

The effect of differences in pH on the properties of starch pastes has been studied by a number of investigators using a single viscosity test. Among these are Porst and Moskowitz (1923), Ripperton (1931), and Richardson and Waite (1933). More recently, Anker and Geddes (1944) determined the effect of pH on buffered wheat starch pastes in the range from pH 5.2 to 6.7, using the amylograph.

The purpose of the present study was to determine the effect of differences of pH, over a wide range, on the pasting characteristics of several commercial corn starches. The pH was adjusted to the de-

sired value by adding small amounts of dilute hydrochloric acid or sodium hydroxide solutions to the starch slurry just preceding the viscosity test. This method was followed in order to approximate the commercial method of neutralizing starches after their modification, and to avoid the introduction of the ions contained in buffers. Such ions may have important effects on viscosity, as shown by the fact that results of tests at the same pH with different buffers vary considerably (Richardson and Waite, 1933; Anker and Geddes, 1944).

Table III, in which the results are summarized, shows the effects of differences in pH on the paste characteristics of the starches, and

TABLE III

EFFECT OF pH

pH	Temperature of initial viscosity rise	Temperature at maximum viscosity	Maximum viscosity	Viscosity after 20 minutes	Breakdown	Ratio $\frac{\text{20 Min. n}}{\text{Maximum n}}$
	°C	°C	G-Cm	G-Cm	G-Cm	
5% UNMODIFIED CORN STARCH						
3	82	89.5	148	128	20	0.87
4	82	89.5	150	150	0	1.00
5	83	90	152	152	0	1.00
6	84	90	150	150	0	1.00
7	83	89.5	150	150	0	1.00
8	79	88	150	140	10	0.93
9	72	84.5	158	96	56	0.61
8% ACID-MODIFIED CORN STARCH, 40 FLUIDITY						
3	72.5	84.5	114	50	64	0.44
4	73	86	114	64	50	0.56
5	72.5	86.5	112	82	30	0.73
6	72	86.5	116	82	34	0.71
7	71	84.5	120	57	63	0.48
8	71	80	118	26	92	0.22
9	69.5	76	138	4	134	0.03
25% ACID-MODIFIED CORN STARCH, 90 FLUIDITY						
3	70.5	77	190	42	148	0.22
5	70	78	196	43	153	0.22
6	69	78	228	52	176	0.23
8	69	79	194	51	143	0.26
9	69	79	156	26	130	0.17
20% OXIDIZED CORN STARCH, 90 FLUIDITY						
3	58	71.5	508	8	500	0.02
5	56	71.5	520	16	504	0.03
6	57	70	440	10	430	0.02
8	56	69.5	170	8	162	0.05
9	56	65.5	60	4	56	0.06

indicates the need for control of pH when comparative studies of starches are made. It will be observed that differences in pH have quite different effects on the various starches. With unmodified and acid-modified corn starch of 40 fluidity, the temperature of initial viscosity rise and of the maximum viscosity are markedly changed. With the acid-modified starch of 90 fluidity these temperatures are not altered appreciably, and with the oxidized starch only the temperature of the maximum at pH 9 is very different from the others.

The effect on maximum viscosity is, however, much more pronounced with the acid-modified starch of 90 fluidity and the oxidized starch. With unmodified and 40 fluidity corn starches the maximum viscosity is rather constant except at pH 9, where it shows an increase. The more highly modified starches, on the other hand, show highest maximum viscosity at pH 6 for 90 fluidity acid-modified starch, and pH 5 for the oxidized starch.

The ratio of the viscosity after 20 minutes' cooking to that at the maximum gives a measure of the relative breakdown in starch viscosity. The lower the ratio, the greater has been the breakdown. Unmodified corn starch does not shown breakdown within 20 minutes between pH 4 and 7. Acid-modified corn starch of 40 fluidity shows the least breakdown at pH 5 to 6. The two highly modified starches break down to a great extent over the entire range, and the differences shown do not appear significant.

A given degree of alkalinity is more effective in changing the viscosity characteristics of these starches than an equal degree of acidity. This is to be expected since cold sodium hydroxide is an excellent swelling agent for starch, and hot alkali causes rapid chemical decomposition.

Effect of Defatting Corn Starch

Since the discovery by Schoch (1938, 1942) of the method for defatting starch by use of 85% methanol, defatted cereal starches have been used for fundamental studies by a number of investigators (Schoch, 1942, Whistler and Hilbert, 1945, Kerr, 1945). The literature contains a few references to indicate that removal of fat alters some paste properties (Schoch, 1942, Whistler and Hilbert, 1944). Caesar (1944) presents consistometer curves which show differences between defatted and normal unmodified corn starch. It therefore seemed of interest to measure the effect of defatting several of the commercial modified corn starches, as well as unmodified corn starch, by means of the Corn Industries Viscometer.

The starches selected were defatted by the method of Kerr (1943) and the remaining fat was determined by the method of Taylor and

Nelson (1920). A summary of the data obtained from the viscosity curves is presented in Table IV, and the complete viscosity curves for commercial and defatted unmodified corn starch are given in Figure 6.

TABLE IV

COMPARISON OF VISCOSITY OF DEFATTED AND COMMERCIAL CORN STARCHES

Starch	pH	Fat	Temperature of initial viscosity rise	Temperature of maximum viscosity	Maximum viscosity	Viscosity after 20 minutes	Ratio $\frac{20 \text{ Min. } n}{\text{Maximum } n}$
		%	°C	°C	G-Cm	G-Cm	
5%, UNMODIFIED							
Commercial	6.4	0.63	82	90[1]	164	164	1.0
Defatted	6.7	0.17	71	90[1]	87	87	1.0
8%, 20 FLUIDITY							
Commercial	6.9	—	71	84	420	264	0.63
Defatted	6.7	0.22	68	79	403	192	0.48
8%, 40 FLUIDITY							
Commercial	6.0	—	71	84.5	158	84	0.53
Defatted	6.0	0.18	71	77	113	33	0.30
9%, 60 FLUIDITY							
Commercial	6.5	—	71	81	154	74	0.48
Defatted	6.4	0.20	71	75.5	154	39	0.25
25%, 90 FLUIDITY							
Commercial	5.2	—	68	75.5	200	69	0.35
Defatted	5.6	0.21	67	77	156	99	0.63
21%, OXIDIZED, 90 FLUIDITY							
Commercial	8.8	—	63	74	136	60	0.44
Defatted	8.2	0.16	57	64	54	21	0.39

[1] The original unmodified starch reached the maximum viscosity after 4 minutes' cooking at 90°, 19 minutes from the start of the test. The defatted sample reached the maximum when the paste reached 90°, 15 minutes after the start of the test.

Marked differences are shown throughout the curves. Defatted starches generally gave the initial viscosity rise at a lower temperature than the corresponding undefatted sample, although in the case of 40 and 60 fluidity acid-modified starches no difference in this property appeared, and the greatest difference was shown by unmodified corn starch. In all cases except the 90 fluidity acid-modified starch, the

maximum viscosity occurred sooner, or at a lower temperature, when the starch was defatted.

Defatting had the effect of lowering the maximum viscosity of unmodified corn starch to about half that of the original starch. Acid-modified starches of 20, 40, and 90 fluidity showed lower maximum viscosities when defatted, although in no case was the difference as great as that of unmodified starch. The greatest effect on maximum viscosity was given by the oxidized starch. In this case the defatted sample had a maximum viscosity only four-tenths that of the original starch.

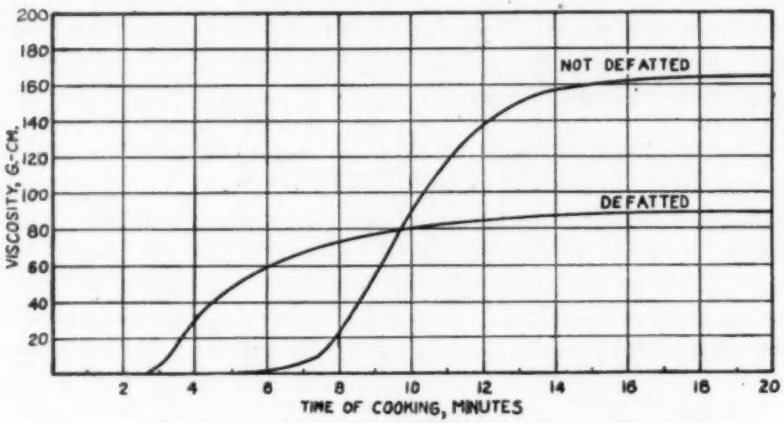

Fig. 6. Effect of defatting on the viscosity of 5% paste of unmodified corn starch.

In all except 90 fluidity acid-modified starch, the defatted sample had a lower viscosity at the end of the 20-minute cooking period than the original starch.

When the relative paste breakdown is studied by means of the ratio of the viscosity at 20 minutes to that at the maximum, it is found that breakdown of defatted starches is proportionately larger than that of the undefatted samples, except for unmodified corn starch which showed no breakdown during the period of the test, and for the 90 fluidity acid-modified starch.

A further difference observed is that defatted starches are much more difficult to suspend in water. In order to prepare pastes of defatted starches free from lumps it was necessary to allow the starch-water slurry to stand at room temperature for about an hour before making the test.

Summary

The Corn Industries Viscometer is described, and is presented as an instrument suitable for research, sales service, and control testing

of starch products. The necessity is shown for a viscosity curve in place of the single viscosity test usually made, if starches are to be characterized adequately.

Results of several studies made with the Corn Industries Viscometer are included as suggesting types of investigations in which it has proved of value. The study of the effect of different heating rates on pastes of unmodified corn starch shows that rapid heating causes an earlier initial viscosity rise, decreases the cooking time required to reach the maximum viscosity, and that it causes a higher viscosity throughout the cooking period.

Differences in the final paste temperature are found to alter the maximum viscosity of a starch and also its rate of paste breakdown. Higher temperatures favor rapid breakdown, and a small temperature difference may result in very different viscosity characteristics.

The findings of other investigators with regard to the effect of changes of concentration on the viscosity of starches are confirmed. The linear relationship found by Anker and Geddes (1944) for the logarithm of maximum viscosity vs. logarithm of starch concentration has been confirmed and extended to other starches, including modified corn starches.

It has been found that differences in pH alter the temperature of initial viscosity rise and that of the maximum viscosity, and also change the maximum viscosity and the rate of paste breakdown. This has been found for modified as well as unmodified corn starch.

Defatted corn starch is shown to be markedly different from normal corn starch throughout its viscosity curve. For unmodified and modified corn starches in general, defatting has been found to lower the temperature of initial viscosity rise, the temperature of the maximum viscosity, and the value of the maximum viscosity. The rate of breakdown of pastes of defatted corn starches is generally greater than that of the corresponding commercial starch. Defatted starches are more difficult to suspend in cold water than the commercial samples.

Acknowledgment

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Literature Cited

Anker, C. A., and Geddes, W. F.
1944 Gelatinization studies upon wheat and other starches with the amylograph. Cereal Chem. **21**: 335–360.
Barham, H. N., Wagoner, J. A., and Reed, G. N.
1942 Viscosity of starches. Ind. Eng. Chem. **34**: 1490–1495.

Buel, H.
1912 A study of some of the physical properties of starches. 8th Intern. Congr. Appld. Chem., Washington and New York, Orig. Comm. **13**: 63–76.
Caesar, G. V.
1932 Consistency changes in starch pastes. Ind. Eng. Chem. **24**: 1432–1435.
1944 Chemistry and Industry of Starch. R. W. Kerr. Academic Press, Inc. New York. Pp. 178–187.
——, and Moore, E. E.
1935 Consistency changes in starch pastes. Ind. Eng. Chem. **27**: 1447–1451.
Glabe, E. F.
1939 Some factors influencing the viscosity of rice flour suspensions. Cereal Chem. **16**: 661–665.
Katz, J. R.
1938 Viscosity of Starch Pastes. Textile Research **9**: 69–78.
Kerr, R. W.
1943 Defatting procedure for corn starch. Cereal Chem. **20**: 299–301.
1945 The heterogeneity of amylose and amylopectin. Arch. Biochem. **7**: 377–392.
Kesler, C. C., and Bechtel, W. G.
1947 Recording viscometer for starches. Analytical Chem. **19**: 16–21.
Porst, C. E. G., and Moskowitz, M.
1923 Plastometer tests on alkaline thin-boiling cornstarches. Ind. Eng. Chem. **15**: 166–170.
Richardson, W. A., and Waite, R.
1933 The flow of starch pastes (iii). J. Textile Inst. **24**: T383–417.
Ripperton, J. C.
1931 Physicochemical properties of edible canna and potato starches. Hawaii Agr. Expt. Sta. Bul. 63.
Schoch, T. J.
1938 Absence of combined fatty acid in cereal starches. J. Am. Chem. Soc. **60**: 2824.
1942 Non-carbohydrate substances in cereal starches. J. Am. Chem. Soc. **64**: 2954–2956.
Taylor, T. C., and Nelson, J. M.
1920 Fat associated with starch. J. Am. Chem. Soc. **42**: 1726–1738.
Whistler, R. L., and Hilbert, G. E.
1944 Extraction of fatty substance from starch. J. Am. Chem. Soc. **66**: 1721–1722.
1945 Separation of amylose and amylopectin by certain nitroparaffins. J. Am. Chem. Soc. **67**: 1161–1165.

A NUTRITIONAL STUDY OF THE FORTIFICATION OF GRAHAM-TYPE CRACKERS WITH SOY GRITS, CALCIUM, AND SEVERAL VITAMINS

SHIRLEY C. CARLSON, ELIZABETH C. HERRMANN, R. M. BOHN, and J. W. HAYWARD

Departments of Nutrition and Food Research, Archer-Daniels-Midland Company, Minneapolis 2, Minnesota

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During recent years there has been considerable interest in the fortification of commonly used low-cost foods with protein, minerals, and vitamins as a means of bettering the nutritional status of people in all economic brackets. A widely used food for children, such as the graham cracker, affords an excellent opportunity for dietary supplementation.

This paper describes the fortification of a graham-type cracker dough and the consequent nutritional effects on the finished cracker. The fortification was accomplished by modifying the accepted graham cracker formula as follows: (1) 30% defatted soy grits were substituted for the 30% graham flour, and (2) calcium, riboflavin, niacin, carotene, and vitamin D were added to the dough (see formula). A palatable soy cracker, fortified with calcium and several vitamins, and resembling the graham cracker in appearance, resulted. We have called it a fortified graham-type soy cracker.[1]

The nutritional contribution of the protein in 3% to 5% of soy flour to white flour protein has been treated in a previous paper (Carlson, Hafner, and Hayward, 1946). The present study evaluates the supplementary effect of the protein in 30% of soy grits for white flour protein. The marked improvement in both quantity and nutritional quality of the protein in white flour effected by the addition of 20–30% soya has been shown by a number of investigators, including Hove, Carpenter, and Harrel (1945), and Bricker, Mitchell, and Kinsman (1945). Horvath (1938) has reviewed much of the early work on this subject.

The above experiments demonstrate the supplementary value of soy protein for white flour protein in mixtures of the flours or in bread. Supplementation in graham crackers, however, is a different problem, since the pH in the finished crackers is 7.5 to 9, whereas the final pH of bread is 5 to 6. It was thought that the greater alkalinity of graham crackers might result in considerably greater amino acid destruction during baking than would occur in bread.

[1] Hereafter in this paper the fortified graham-type soy cracker will be referred to as the graham-type soy cracker. The flour portion of this cracker consisted of 30% soy grits and 70% white flour.

Light and Frey (1943) have demonstrated that the supplementary value of any protein for wheat protein is due to its lysine and valine content. Soy grits, because of their high protein content, increase the total percentage of amino acids when substituted for the graham flour in a graham-type cracker. In addition to raising the protein content of the crackers, soy protein has a specific supplementary value for white flour protein because it contains a relatively large amount of lysine, 5.8%, in contrast to 2.0% in white flour protein. The following table shows the increased lysine and valine content of graham-type soy crackers over regular graham crackers:

	Protein (N×6.25) in ingredient	Lysine in protein (16% N basis)	Lysine contributed by each ingredient	Total lysine in crackers	Valine in protein (16% N basis)	Valine contributed by each ingredient	Total valine in crackers
	%	%	%	%	%	%	%
GRAHAM-TYPE SOY CRACKER:							
Soy grits	51.2	5.8	0.57		4.2	0.41	
White flour	10.0	2.0	0.10		4.2	0.19	
				0.67			0.60
REGULAR GRAHAM CRACKER:							
Graham flour	11.9	2.7	0.06		4.5	0.10	
White flour	10.0	2.0	0.10		4.2	0.19	
				0.16			0.29

The amino acid values were calculated from data supplied by R. J. Block (1946) on the composition of white, whole wheat, and soy flours. Due to losses in baking the actual percentages of these two amino acids, particularly lysine, may be less than calculated. However, these losses should be proportional in the two crackers.

When 30% graham flour was replaced by 30% soy grits in graham-type soy crackers, the protein was increased so that one ounce (3 to 5 crackers) provided $\frac{1}{16}$th of a 10–12 year old child's daily protein allowance (National Research Council, 1943). In order to supply corresponding amounts of other selected nutrients, calcium and a number of vitamins were added to the dough.[2] The basis for, and nutrient levels achieved by, fortifying the graham-type soy cracker are shown in the table at top of page 217.

No thiamine was added to the cracker because of the assumed instability of this vitamin under the baking conditions of high oven temperature and alkalinity. Commercial graham crackers have a final pH of 7.5 to 9.0.

[2] The inclusion of vitamin A in the graham-type soy crackers is not in accord with the recommendations of the National Research Council (1944), since vitamin A is not a characteristic ingredient of cereal foods. The council permits the addition of vitamin D and calcium as optional ingredients.

	Daily recommended allowance for 10–12 year old child (Natl. Research Council, 1943)	¼ daily recommended allowance	Nutrients per ounce of graham-type soy cracker
Protein	70 g	4.4 g	4.3 g
Calcium	1.0 g	62.5 mg	62.5 mg
Riboflavin	1.8 mg	112.5 μg	119 μg
Niacin	12 mg	750.0 μg	750 μg
Vitamin A	4500 I.U.	281.3 I.U.	284.4 I.U.
Vitamin D	800 I.U.	50.0 I.U.	50 I.U.

In this paper, the nutritional effect of fortifying graham-type crackers, as described in the table above, is studied. Biological tests demonstrating the effect on the nutritional quality of the protein, as well as the effect on the crackers as the only source of food, are described.

Materials and Methods

Preparation of the Crackers. The graham-type soy crackers and regular graham crackers were produced on standard equipment at a commercial bakery under the supervision of a man experienced in practical cracker production. The following formulas were used:

	Graham-type soy cracker	Regular graham cracker
Cracker sponge flour	700 lb.	700 lb.
Graham flour	—	300 lb.
Soy grits, defatted type[3]	300 lb.	—
Sugar	250 lb.	250 lb.
Invert syrup	50 lb.	50 lb.
Molasses	50 lb.	50 lb.
Shortening	125 lb.	125 lb.
Soda	10 lb.	10 lb.
Salt	10 lb.	10 lb.
Ammonia	10 lb.	10 lb.
Water	250 lb.	250 lb.
Riboflavin	1.8 mg/lb.	
Niacin	12.0 mg/lb.	
Carotene in oil[4]	1.9 g/lb.	
Irradiated yeast[5]	33.0 mg/lb.	
Dicalcium phosphate	2.7 g/lb.	

A hot dough was made in both cases, and the dough was handled and baked as for regular graham crackers. The graham-type soy dough ran perfectly. It rolled easily, sheeted well, and gave no trouble in cutting, peeling, or baking.

[3] Hexane-extracted soy flakes cooked for optimum nutritive quality and best flavor. The size of the grits was such that 100% passed through a No. 16 screen and 100% remained over a No. 30 screen.
[4] The 1.9 g of the carotene in oil preparation provided 4500 I.U. of carotene.
[5] The 33 mg of irradiated yeast provided 800 I.U. of vitamin D.

The crackers were ground to about 80 mesh for incorporation into the diets. Table I contains the analysis of the two types of crackers and nonfat dry milk solids as they were used in the diets. The analyses were made according to either A.O.A.C. approved or modifications of tentative methods (A.O.A.C., 1945). The methods for calcium and phosphorus were titrametric, for iron and carotene, colorimetric, and for thiamine and riboflavin, fluorometric.

TABLE I

ANALYSIS OF TEST MATERIALS

	Graham-type soy cracker	Regular graham cracker	Nonfat dry milk solids
Protein, % (N×6.25)	15.5	7.5	34.8
Moisture, %	7.3	5.80	5.2
Fat, %	9.3[1]	11.7[1]	0.6[2]
Crude fiber, %	0.9	0.3	—
Ash, %	2.6	1.8	—
Calcium, %	0.25	0.06	—
Phosphorus, %	0.38	0.22	—
Iron, μg/g	33.8	14.8	—
Thiamine, μg/g	0.57	0.61	—
Riboflavin, μg/g	9.34	4.07	—
Carotene, μg/g	3.37	2.52	—

[1] Acid hydrolysis.
[2] Rohrig tube method of Roese Gottlieb.

Feeding and Experimental Procedure. Graham-type soy crackers were compared with regular graham crackers by the use of two biological techniques:

(1) a 26-week growth test in which the crackers were fed as the sole source of food.

(2) an 8-week protein assay in which the materials tested were incorporated as the sole source of protein into supplemented diets of equal protein content.

Sixty male weanling albino rats of the Sprague-Dawley strain, 22–25 days old and weighing 40–50 g each, were distributed at random into 10 lots of 6 rats each. They were individually caged in an air-conditioned room where the temperature was maintained at 75° ± 2° F at a relative humidity of 50–70%. The animals were given free access to water. The composition of the diets is given in Table II.

A detailed description of the two biological techniques follows:

(1) THE 26-WEEK GROWTH TEST (LOTS 1, 2, 3, AND 4). The two crackers were fed ad libitum as the sole source of all nutrients (Lots 1 and 2) and no attempt was made to equalize the protein level. For Lots 3 and 4, vitamins and minerals were added to the graham-type soy cracker diet in addition to those which had been used to fortify

TABLE II

COMPOSITION OF DIETS

	26-week test				8-week protein assay		
	Lot 1	Lot 2	Lot 3	Lot 4	Lots 5 & 8	Lots 6 & 9	Lots 7 & 10
	Parts	*Parts*	*Parts*	*Parts*	*Parts*	*Parts*	*Parts*
Regular graham cracker	100.0	—	—	—	75.5	—	—
Graham-type soy cracker	—	100.0	98.0	97.0	—	37.7	—
Nonfat dry milk solids[1]	—	—	—	—	—	—	16.0
Minerals[2]	—	—	—	2.0	4.0	4.0	4.0
Tricalcium phosphate	—	—	2.0	—	—	—	—
Cod liver oil[3]	—	—	—	—	2.0	2.0	2.0
Liver fraction E[4]	—	—	—	1.0	1.0	1.0	1.0
Sucrose	—	—	—	—	10.0	10.0	10.0
Celluration[5]	—	—	—	—	2.5	2.5	2.5
Soybean oil	—	—	—	—	—	5.3	8.7
Corn starch	—	—	—	—	5.0	37.6	55.4
Vitamins, mg/100 g diet							
Thiamine	—	—	.300	.400	.400	.400	.400
Choline	—	—	.200	.200	.200	.200	.200
Riboflavin	—	—	—	.400	.400	.400	.400
Pyridoxine	—	—	—	.400	.400	.400	.400
Niacin	—	—	—	2.5	2.5	2.5	2.5
Para-aminobenzoic acid	—	—	—	7.5	7.5	7.5	7.5
Calcium pantothenate	—	—	—	1.1	1.1	1.1	1.1
Inositol	—	—	—	20	20	20	20
Vitamin K (synthetic)	—	—	—	.050	.050	.050	.050
Totals (excluding vitamins)	100.0	100.0	100.0	100.0	100.0	100.0	100.0

[1] Spray dried, obtained from Twin City Milk Producers Association, Minneapolis.
[2] Osborne-Mendel-Wesson Salt Mixture (Wesson, 1932) modified to contain 0.13 g of $ZnSO_4 \cdot 7\ H_2O$ per kilogram.
[3] Upjohn's super D, with 2.5 mg alpha tocopherol added per 2 ml of oil.
[4] Wilson Laboratories, Chicago.
[5] Processed rice hulls, analyzing approximately 65–70% crude fiber and 0.0% protein, purchased from Fisher Scientific Co., Chicago.

the crackers before baking. The animals were fed their respective diets for 26 weeks unless they died before this time. This test was evaluated by livability and weight gains of the animals.

(2) THE 8-WEEK PROTEIN ASSAY (LOTS 5–10). In the protein assay, nonfat dry milk solids were used as a control. Lots 5, 6, and 7 were fed ad libitum and food intake was equalized among Lots 8, 9, and 10. Nitrogen storage determinations were made. The assay

procedure for ad libitum and equalized feeding tests and the method of determining nitrogen storage have been described in a previous publication (Carlson, Hafner, and Hayward, 1946). The test materials were incorporated into purified diets complete with respect to all nonprotein dietary essentials. The protein level of the diets was equalized at 5.7% because the low protein content of regular graham crackers prevented the formulation of a supplemented diet of higher protein content. Values for protein efficiency [6] and nitrogen storage efficiency [7] were calculated and used along with weight gains to evaluate this protein assay.

Results and Discussion

The 26-Week Growth Test. The experimental data, as contained in Table III, show that graham crackers fortified before baking with soy grits, calcium, and several vitamins have markedly superior nutritional quality to regular graham crackers. All animals fed regular graham crackers as the sole source of food (Lot 1) had lost an average of 7.7 g by the end of the first 8 weeks, and died between the 9th and 20th weeks of the test. In contrast, the rats fed graham-type soy crackers alone had made an average gain of 156.6 g by the end of the first 8 weeks, or an average daily gain of 2.8 g during this period. These rats were alive and healthy at the end of 26 weeks.

Further supplementation of regular graham crackers by the addition of a complete vitamin-mineral supplement to the diet did not improve the very poor growth-promoting quality of these crackers. The animals fed regular graham crackers plus a complete vitamin-mineral supplement (Lot 5) had lost about the same amount of weight at 8 weeks as those fed regular graham crackers without the supplement (Lot 1). Since no growth response was obtained from regular graham crackers by adding vitamins and minerals, it appears that the protein deficiency of these crackers must be corrected before any nutritional improvement can be shown.

The graham-type soy crackers, fortified before baking with calcium, riboflavin, niacin, carotene, and vitamin D, were apparently a satisfactory source of vitamins and minerals for the rat. Animals fed graham-type soy crackers as the sole source of food (Lot 2) grew as well as those fed the same crackers plus a dietary supplement of either calcium, thiamine, and choline (Lot 3), or a complete vitamin-mineral mixture (Lot 4).

[6] The term "protein efficiency," as used in this paper, refers to the grams gain in weight per gram protein eaten, sometimes called the nutritive value of protein.

[7] The term "nitrogen storage efficiency" is used in this paper to designate the grams gain in body nitrogen per gram nitrogen eaten.

TABLE III

SUMMARY OF EXPERIMENTAL DATA

Lot No.	Test material[1]	Dietary vitamin-mineral supplement	Dietary protein level (N ×6.25)	Type of feeding	8-week protein data					26-week data	
					Protein consumed	Weight gain	Protein efficiency[2]	Body nitrogen gain	Nitrogen[3] storage efficiency	Weight gain	Deaths
			%		g	g		g		g	
26-WEEK GROWTH TEST											
1	Regular graham cracker	None	7.5	Ad lib	15.56	−7.7	0	—	—	—	6
2	Graham-type soy cracker	None	15.5	Ad lib	100.23	156.6	1.56	—	—	307.8	0
3	Graham-type soy cracker	Ca, B_1, Choline	15.3	Ad lib	89.6	133.9	1.49	—	—	269.1	0
4	Graham-type soy cracker	Complete	15.1	Ad lib	83.4	117.6	1.41	—	—	271.5	0
8-WEEK PROTEIN ASSAY											
5	Regular graham cracker	Complete	5.7	Ad lib	11.82	−7.4	0	−0.17	0	—	—
6	Graham-type soy cracker	Complete	5.7	Ad lib	25.30	28.2	1.11	0.77	0.18	—	—
7	Nonfat dry milk solids	Complete	5.7	Ad lib	27.86	65.2	2.34	1.83	0.40	—	—
8	Regular graham cracker	Complete	5.7	Equalized	11.27	−6.7	0	−0.14	0	—	—
9	Graham-type soy cracker	Complete	5.7	Equalized	11.27	2.4	0.21	0.15	0.07	—	—
10	Nonfat dry milk solids	Complete	5.7	Equalized	11.27	9.4	0.83	0.32	0.17	—	—

[1] Graham-type soy crackers were fortified before baking with calcium, riboflavin, niacin, carotene, and yeast; regular graham crackers were not fortified and were made by a commercial formula.
[2] Grams gain in weight per gram protein eaten, sometimes called nutritive value of the protein.
[3] Grams gain in body N per gram N eaten.

This was surprising, particularly because of the low level of thiamine in the graham-type soy crackers (57 μg/100 g). These crackers, when fed alone as in Lot 2, supplied only 7.2 μg of thiamine daily to each animal. This amount is slightly below the minimum thiamine requirement of the rat for growth, which is about 80–100 μg/100 g of food, according to Arnold and Elvehjem (1938). Sure (1938) states 10 μg are required daily for normal growth.

It is possible that dextrin, formed from the starch during baking, may have exerted a thiamine-sparing action on our low thiamine diet. Emerson and Obermeyer (1944), Guerrant *et al.* (1935 and 1937), and Krehl *et al.* (1946) found that dextrin increased the intestinal synthesis of B-vitamins, including thiamine, and hence could exert a thiamine-sparing action on low thiamine diets.

The 8-Week Protein Assay. Our results indicate that the most critical deficiency of regular graham crackers is protein, rather than vitamin or mineral in nature. Data on the last 6 lots in Table III show that this deficiency was qualitative as well as quantitative, and that it was corrected to a large extent by the substitution of soy grits for the graham flour, as accomplished in the graham-type soy crackers.

The nutritional quality of the graham-type soy cracker protein was significantly superior to that of regular graham cracker protein in all comparisons. Under conditions of equalized, as well as ad libitum food intake, the protein efficiencies and nitrogen storage efficiencies for graham-type soy crackers (Lots 6 and 9) were significantly higher than those for regular graham crackers (Lots 5 and 8). All differences were highly significant statistically (odds greater than 99:1). All rats fed regular graham crackers as the only source of dietary protein (Lots 5 and 8) lost weight and body nitrogen during the 8-week test period. On the other hand, animals fed graham-type soy crackers as the only source of dietary protein (Lots 6 and 9) gained weight and body nitrogen. The control animals fed nonfat milk solids (Lots 7 and 10) also gained weight and body nitrogen.

Results of the equalized feeding and the ad libitum protein assay agreed well, in spite of the severe conditions imposed on the animals in the equalized feeding assay. The abnormally low protein efficiency and nitrogen storage efficiency values in the equalized feeding assay were the result of the low protein intake of only 0.2 g per day. For maximum protein efficiency, Barnes *et al.* (1945) recommend a protein intake of 0.9 g per day. The low protein intake in our equalized protein assay resulted from the fact that (1) all animals were restricted to the small amount of food consumed daily (average 3.5 g) by those on the regular graham cracker diet and (2) the dietary protein level was only 5.7%.

Evidence that the protein level, even in the ad libitum protein assay, was not optimum for graham-type soy crackers is shown by the fact that the protein efficiency of these crackers was increased from 1.11 to 1.41 when the protein level was increased from 5.7% (Lot 6) to 15.1% (Lot 4). This difference was significant (odds 19:1). The protein level for nonfat milk solids was close to its optimum in the ad libitum assay, since its protein efficiency at a 5.7% level, 2.34, was approximately the same as its protein efficiency at a 10% level, 2.36.[8] Therefore, because the optimum protein level for nonfat milk solids is lower than that for graham-type soy crackers, the difference in protein efficiency is exaggerated by a comparison at the low level of dietary protein used in this assay.

These experiments show that soy protein markedly supplemented wheat protein under the conditions used in making graham crackers, i.e., pH 7.5 to 9 and intense heat during baking. Therefore, fortification of graham crackers with soy grits is practical from a nutritional point of view.

Summary

The nutritional effect of fortifying graham crackers by the substitution of 30% soy grits for the graham flour and by the addition of calcium, riboflavin, niacin, carotene, and vitamin D was studied. The palatable graham-type soy cracker which resulted, protein 15.5%, was compared with regular graham crackers, protein 7.5%, using two biological techniques: a 26-week growth test in which the crackers were fed to rats as the sole source of food, and an 8-week protein assay which included nitrogen storage determinations, and ad libitum and equalized feeding.

Graham-type soy crackers, fortified before baking, promoted growth at the rate of 2.8 g per day the first 8 weeks of the 26-week growth test without the addition of any dietary vitamin or mineral supplement. All six rats were alive and healthy at the end of 26 weeks, and evidence that the graham-type soy crackers exerted a thiamine-sparing action is given. However, all six animals fed regular graham crackers lost weight the first 8 weeks and died at the end of 9 to 20 weeks.

Our studies indicate that regular graham crackers are deficient primarily in protein, rather than in vitamins or minerals. All tests for the nutritional quality of the protein showed graham-type soy crackers to be markedly superior to regular graham crackers. Apparently the baking conditions for crackers, i.e., slightly alkaline pH and high oven temperature, did not impair the supplementary value of soy protein for white flour protein.

[8] This value is the mean protein efficiency for six separate assays of the same product. Results of these assays are unpublished. Standard error of mean =0.023.

Acknowledgment

The authors are indebted to Colonel C. W. Griggs and his co-workers of Griggs Cooper and Company, St. Paul, for baking the crackers used in these experiments, and to Fred H. Hafner, a former member of our research staff, for his assistance in the initiation and planning of the nutritional studies. We also wish to express our appreciation to the following people of our company: Royce E. Anderson, for the analyses of materials, and Dorothy V. Zellers, for her help with various phases of the work.

Literature Cited

Arnold, A., and Elvehjem, C. A.
1938 Studies on the vitamin B_1 requirements of growing rats. J. Nutrition **15**: 429–443.

Association of Official Agricultural Chemists
1945 Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists. 6th ed.

Barnes, R. H., Maack, J. E., Knights, M. J., and Burr, G. O.
1945 Measurement of the growth-promoting quality of dietary protein. Cereal Chem. **22**: 273–286.

Block, R. J.
1946 Personal communication.

Bricker, M., Mitchell, H. H., and Kinsman, G. M.
1945 The protein requirements of adult human subjects in terms of the protein contained in individual foods and food combinations. J. Nutrition **30**: 269–283.

Carlson, S. C., Hafner, F. H., and Hayward, J. W.
1946 Effect of soy flour and nonfat dry milk solids in white bread on the nutritional quality of the protein as measured by three biological methods. Cereal Chem. **23**: 305–317.

Emerson, G. A., and Obermeyer, H. G.
1944 Thiamine utilization of rats maintained on diets containing dextrimaltose. Proc. Soc. Exptl. Biol. Med. **57**: 216–221.

Guerrant, N. B., Dutcher, R. A., and Tomey, L. F.
1935 The effect of the type of carbohydrate on the synthesis of the B vitamins in the digestive tract of the rat. J. Biol. Chem. **110**: 233–243.

——, ——, and Brown, R. A.
1937 Further studies concerning the formation of the B vitamins in the digestive tract of the rat. J. Nutrition **13**: 305–315.

Horvath, A. A.
1938 The nutritional value of soybeans. Am. J. Digestive Diseases **5**: 177–183.

Hove, E. L., Carpenter, L. E., and Harrel, C. G.
1945 The nutritive quality of some plant proteins and the supplemental effect of some protein concentrates on patent flour and whole wheat. Cereal Chem. **22**: 287–295.

Krehl, W. A., Sarma, P. S., and Elvehjem, C. A.
1946 The effect of protein on the nicotinic acid and tryptophane requirement of the growing rat. J. Biol. Chem. **162**: 403–411.

Light, R. F., and Frey, C. N.
1943 The nutritive value of white and whole wheat breads. Cereal Chem. **20**: 645–660.

National Research Council, Food and Nutrition Board
1943 Recommended Dietary Allowances. Cir. Series 115.

——, Food and Nutrition Board
1944 Enrichment of flour and bread. Bulletin 110.

Sure, B.
1938 Influence of avitaminoses on weights of endocrine glands. Endocrinology **23**: 575–580.

Wesson, L. G.
1932 A modification of the Osborne-Mendel salt mixture containing only inorganic constituents. Science **75**: 339–340.

A SIMPLIFIED PROCEDURE FOR THE DETERMINATION OF BARLEY EXTRACT[1]

W. O. S. MEREDITH

Grain Research Laboratory, Board of Grain Commissioners for Canada, Winnipeg, Manitoba

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The procedure used by Ayre, Sallans, and Anderson (1940) for the determination of barley extract has certain limitations. One of them is that the sample for analysis is ground in a ball mill, which is not standard apparatus in a barley or malt laboratory; ball milling also requires 24 hours for grinding one sample, so that the operation is time consuming and limits the rate of analysis. A further disadvantage is that the extraction procedure involves boiling the mash for 10 minutes. This process requires constant supervision to prevent boiling over and the formation of rings of dried material on the beaker. When a number of determinations are being carried out simultaneously, the procedure becomes unwieldy for one analyst. Later investigations in this laboratory have shown that ball milling, boiling, and centrifuging processes are not necessary. A modified procedure is described in this paper and results obtained with it are compared with those for the old procedure and with malt extract values.

Method

Apparatus: (1) Wiley mill with 1 mm sieve, or other type of mill giving similar grist. (2) Mashing apparatus with stirrers and brass beakers, filter paper, pycnometers, etc., as for determination of malt extract according to *Methods of Analysis*, American Society of Brewing Chemists (1944). (3) Constant temperature bath maintained at 20°C.

Solution: 2.6 g alpha-amylase [2] and 6.5 g malt diastase [3] made into a smooth paste with water and then diluted to 1300 ml 2 to 3 hours before use. This provides sufficient solution for 12 samples.

Grinding: Grind the sample in a Wiley mill through 1 mm sieve, or equivalent mill. Complete grinding is usually achieved in Wiley mill in 3 minutes.

Determination: Weigh 15.0 g ground material (as is) into tared mash beaker. Add 100 ml amylase solution, taking care to mix slowly at first to avoid lumps, and stand overnight (16–18 hours) at 20°C. Add sufficient water to cover stirrer blade if necessary. Place beaker

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[2] Alpha-amylase, special for analytical purposes, a concentrated bacterial enzyme preparation prepared by Wallerstein Laboratories, New York.

[3] Malt diastase, special for analytical purposes, prepared by Wallerstein Laboratories, New York.

in mash bath at 48° to 50°C and set stirrers in motion. Hold at this temperature for 10 minutes. Raise the mash temperature 1° per minute until 75°C is reached and hold at this temperature for 30 minutes. Cool to room temperature (approximately 22°C) within 10–15 minutes and rinse stirrers into beaker. Make up contents of beaker to 135 g by addition of water. Stir this mash thoroughly and pour entire contents on to a funnel provided with specified filter paper (E. & D. No. 509); return first 50 ml filtrate to the filter. Determine specific gravity of the filtrate at 20°C by means of pycnometer as in the A.S.B.C. Methods.

Extract blank: Carry out a blank determination on the amylase solution, mashing as for the barley extract determination, but make up final weight to 128 g. Filter and determine specific gravity of filtrate.

Calculations: Subtract specific gravity of blank in excess of 1 from specific gravity of barley wort. Determine extract yield of barley by reference to appropriate specific gravity and moisture content in *Tables for Extract Determination in Malt and Cereals* published by American Society of Brewing Chemists (1940).

NOTE: The blank determination may be carried out by making up the amylase solution to 135 g. The calculations must then be made according to the method given for Cereal Adjuncts by the American Society of Brewing Chemists (1944). That is, the extract in the amylase solution is subtracted from the "as is" extract content of the barley mash and the resultant value is corrected for barley moisture content. The blank method given above allows for 10% moisture in the barley, and the extract from the amylases is in the same dilution as in the barley mash. This procedure greatly simplifies calculation and is adequate for routine use.

Comparison of Barley Extract Methods

Twenty barley samples that had been subjected to routine malting tests were analyzed for barley extract by the old procedure (Ayre, Sallans, and Anderson, 1940) and by the modified procedure. The results for these determinations and for malt extract are given in Table I. It is obvious that there is fairly good agreement between the two methods and also between each method and malt extract. The modified procedure produces results that are somewhat lower than those for the old method, but the results of the two methods are related to malt extract to about the same degree. The correlation coefficients and standard errors of prediction in Table II bring out this point more clearly. The two methods are not as closely related as one would like when substituting a new method for a standard method. But this case involves the substitution of one prediction method for another. The correlation coefficients between each method and malt extract are practically identical; the errors in predicting malt extract are also similar, and both methods therefore give useful results. There

TABLE I

COMPARISONS OF RESULTS OF TWO BARLEY EXTRACT METHODS AND MALT EXTRACTS FOR TWENTY SAMPLES

Sample	Barley extract		Malt extract
	New method	Old method	
	%	%	%
1	78.8	80.2	79.8
2	76.7	78.0	77.8
3	77.7	79.8	77.7
4	78.8	78.1	77.2
5	76.7	78.2	76.6
6	77.8	79.0	76.3
7	76.1	74.8	76.0
8	75.1	75.7	75.7
9	76.8	78.3	75.4
10	75.3	75.5	74.9
11	76.0	76.3	74.8
12	76.1	77.2	74.6
13	74.2	75.1	74.6
14	76.0	74.6	74.6
15	76.5	76.7	74.2
16	73.9	74.2	74.2
17	74.0	73.7	74.1
18	73.9	75.6	74.0
19	75.6	75.2	74.0
20	74.3	74.2	73.7
Mean	76.0	76.5	75.5

TABLE II

CORRELATION COEFFICIENTS AND STANDARD ERRORS OF PREDICTION AMONG BARLEY EXTRACT METHODS AND MALT EXTRACT

Properties	Correlation coefficient	Standard error of prediction
		% *Ext.*
Malt extract × old method	.81**	0.97
Malt extract × modified method	.80**	0.99
Old method × modified method	.87**	

** Significant to 1% level.

is a slight advantage in favor of the old method in that the standard error of a single determination for it is 0.35% extract as compared with 0.44% for the modified procedure. However, the modified procedure is much more suitable for routine use than is the old method, so that the balance is in favor of the new method.

Literature Cited

American Society of Brewing Chemists
1940 Tables for Extract Determination in Malt and Cereals. Third Ed.
1944 Methods of Analysis. Fourth Revised Ed.
Ayre, C. A., Sallans, H. R., and Anderson, J. A.
1940 Varietal differences in barleys and malts. IX. Carbohydrate fractions of barley and their correlations with total nitrogen and 1000 kernel weight. Can. J. Research **C 18**: 169–177.

A NOTE ON THE VIABILITY OF WHEAT SEEDS

R. WHYMPER [1] and A. BRADLEY [2]

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Continuing our experiments recorded in *Cereal Chemistry* (1934) on the viability of a sample of English wheat that we had stored under many different conditions in 1913, one of which (desiccation) encouraged longevity, one of us (A. B.) has carried out germination tests for the years 1933–1945 on that sample that showed considerable vitality after 14 years. Table I gives the more recent results obtained which, unfortunately owing to the sample being now exhausted, are the last that we can record.

TABLE I

GERMINATION OF ENGLISH WHEAT AFTER 20–32 YEARS OF STORAGE

Year	Age	Germination	Moisture in sample
	Years	%	%
1933	20	82	4.51
1934	21	80	4.46
1935	22	81	4.60
1936	23	76	4.55
1937	24	77	4.59
1938	25	77	4.62
1939	26	73	4.66
1941	28	71	4.60
1943	30	68	4.72
1945	32	69	4.81

Previous to our experiments, the oldest wheat of authentic origin showing any vitality was an Australian wheat mentioned by White (1909) as germinating to the extent of 2% after a period of 16½ years. The results claimed by Count Sternberg (1853) for Egyptian "mummy wheat" several thousands of years old have long been discredited by us and by others before us.

However, Robertson and Lute (1933) referred to a wheat that germinated 98.1% after 4 years with gradual deterioration of 12% in 10 years, while in their own experiments with wheat stored in an unheated room in Colorado a deterioration of 7% in 10 years was observed. By this time our own wheat of the 1913 crop was already 20 years old and still showing 82% germination under drier conditions of storage. Robertson and Lute (1937), continuing their experiments in Colorado, showed the same slow decline in germination for the first 10-year period with a sharp break between the 10th and 12th years, which was not observed in our experiments. The same authors (1939)

[1] The British Arkady Company, Manchester, England.
[2] Peek, Frean and Company, London, England.

recorded a sample of wheat stored in a dry room as still retaining 80% germination after 15 years and only a small loss in activity after 3 years at 57.6% relative humidity, while our own wheat was 25–26 years old with a 77–73% germinative activity. Again, Robertson, Lute, and Kroeger (1943), after repeating previous findings, stated that from the 10th to 15th year their wheat dropped 22.3%, and between the 15th and 20th year 48.4% when, in its 21st year, it germinated 12.8%, our own wheat still showing 68% germination. The moisture content of the seeds was probably responsible for the differences in the two findings, their wheat containing 9.5–11.4% moisture, our wheat 4.3–4.8%.

In 1932, i.e. after 19 years, our wheat was 83% viable, and in 1945, after 32 years of storage, still germinated 69%.

It is worthy of note that, under our experimental conditions, our wheat of a moisture content 4.3–4.8% dropped in germinative power 17% in 19 years and 31% in 31 years, which is about 1% per annum. The slight progressive but not consistent increase in moisture content over the years should also be noted, the increase being probably due to our opening the tubes to withdraw our samples and resealing them after a short exposure to the atmosphere, although in 1937 our notes show that the wheat had to be entirely removed from the tubes to allow a new sealing-neck to be made, an exposure which did not seem either to increase the moisture content appreciably or to impair the germinative power of the seeds.

Calculation shows that our estimate of 50 years for the viability of our wheat seeds in question would likely have been an understatement of fact.

Literature Cited

Robertson, D. W., and Lute, A. M.
1933 Germination of the seed of farm crops in Colorado after storage for various periods of time. J. Agr. Research **46**: 455–462.
1937 Germination of seed of farm crops in Colorado after storage for various periods of time. J. Am. Soc. Agron. **29**: 822–834.
——, ——, and Gardner, R.
1939 Effect of relative humidity on viability, moisture content, and respiration of wheat, oats, and barley seed in storage. J. Agr. Research **59**: 281–291.
——, ——, and Kroeger, H.
1943 Germination of 20-year-old wheat, oats, barley, corn, rye, sorghum, and soybeans. J. Am. Soc. Agron. **35**: 786–795.
Sternberg, C.
1853 Flora, p. 4.
White, J.
1909 The ferments and latent life of resting seeds. Proc. Roy. Soc. (London) **B 81**: 417–442.
Whymper, R., and Bradley, A.
1934 Studies on the vitality of wheat. I. Prolongation of vitality of wheat seeds. Cereal Chem. **11**: 349–360.

BOOK REVIEW

Onderzoekingen Over De Invloed Van L-Ascorbinezuur En Van Verbindingen Met Verwante Structuur Op De Bakkaard Van Meel. By P. R. A. Maltha. 215 pp. AE Kluwer, Deventer, Holland. 1946.

This book comprises the author's thesis for the doctorate degree from the Technical University, Delft, Holland, and deals with investigations on the influence of l-ascorbic acid and related compounds on the baking quality of flour. The purpose of these studies was to determine whether the isomers of l-ascorbic acid and d-ascorbic acid, as well as d-gluco-ascorbic acid, oxytetronic acid, and reductic acid, show a similar effect to the well-known excellent improving influence of l-ascorbic acid on the baking quality of flour.

The writer remarks that no unanimity of opinion exists concerning the manner in which oxidizing agents improve baking quality, whether indirectly by inhibiting the proteolytic enzymes present in dough, or directly by acting upon the gluten proteins.

According to both concepts the oxidation of glutathione is involved. From the viewpoint of the theory of direct action, the oxidized form is unable to bring about inactivation of proteolytic enzymes; according to those who adhere to the theory of direct action, the oxidized glutathione can no longer cause a direct modification of the gluten proteins by reducing their disulfide bridges. For this reason special attention was paid to the possibility of glutathione oxidation by the various substances which were studied. Improving properties were investigated by means of baking tests and extensographic tests. Of all substances investigated, only reductic acid caused an improvement comparable with that of l-ascorbic acid; the effect of the other substances was of little or no importance.

Based on these observations, an explanation is proposed for the different effects of the various compounds tested on baking quality. When these compounds are present in the dough, each of them is oxidized, producing the dehydro form of the acids. Only dehydro-l-ascorbic acid and dehydro-reductic acid are capable, however, of oxidizing glutathione, and this reaction takes place rapidly. The remaining substances are not, or only to a very small extent, capable of bringing about glutathione oxidation, and therefore have only a slight influence on the baking quality.

There is a slight difference between the conduct of l-ascorbic acid and reductic acid. The oxidation of glutathione by means of dehydro-l-ascorbic acid only takes place rapidly in the presence of flour extract; thus, this oxidation process is purely enzymatic in nature. Dehydro-reductic acid, however, can also oxidize glutathione in the absence of flour extract, although the reaction proceeds more rapidly when it is present. This indicates that the latter oxidation process has an enzymatic as well as a chemical character. Similar studies on the action of potassium bromate prove that it is capable of a pure chemical oxidation of glutathione and the presence of flour extract is not of any influence here.

A separate chapter is devoted to a study of the influence of the analogues of l-ascorbic acid on the degradation of proteins by proteolytic enzymes. After investigating various experimental procedures recommended in the literature, the most reliable results were finally obtained by using papain as the proteolytic enzyme, gelatine as the substrate, and determining the degree of protein hydrolysis by means of a simplified formol titration. In this way data were obtained which fully confirmed the concept that the proteolysis of proteins, activated by glutathione, is vigorously checked by dehydro-l-ascorbic acid, if the mixture also contains flour dehydrases. Whereas the presence of these enzymes is desirable for the activation of glutathione by dehydro-reductic acid, it is not at all necessary when using potassium bromate. It was moreover proved that dehydro-l-ascorbic acid in dough does not directly attack the proteinases, or at least only to a very small extent, but it does attack the activator of glutathione accompanying this enzyme.

Although these researches do not settle the question whether the improvement in baking quality, which is brought about by the addition of small quantities of certain chemicals, is due to a direct action of glutathione on the proteins of flour, or to an indirect action by eliminating the activating effect of glutathione on the proteinases of flour—the nature of the results favors the latter concept.

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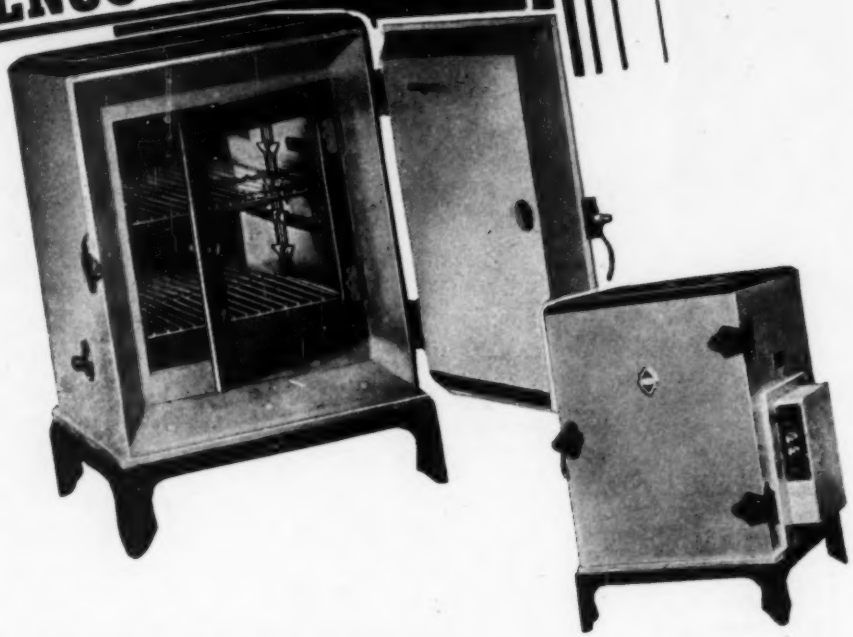

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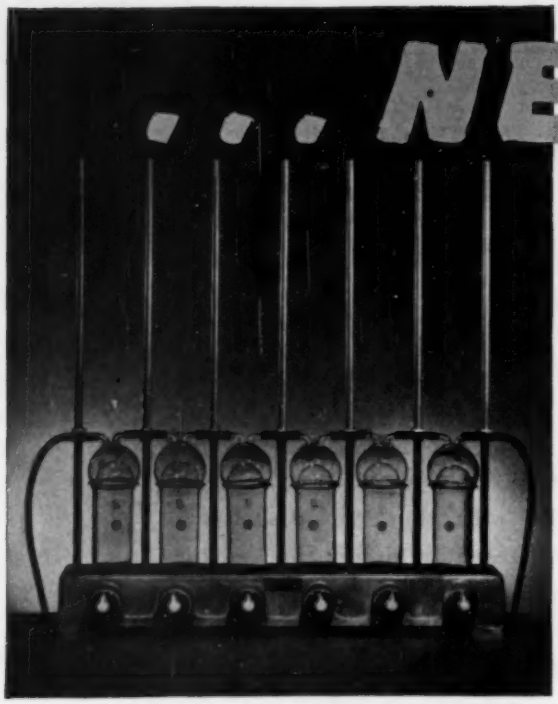

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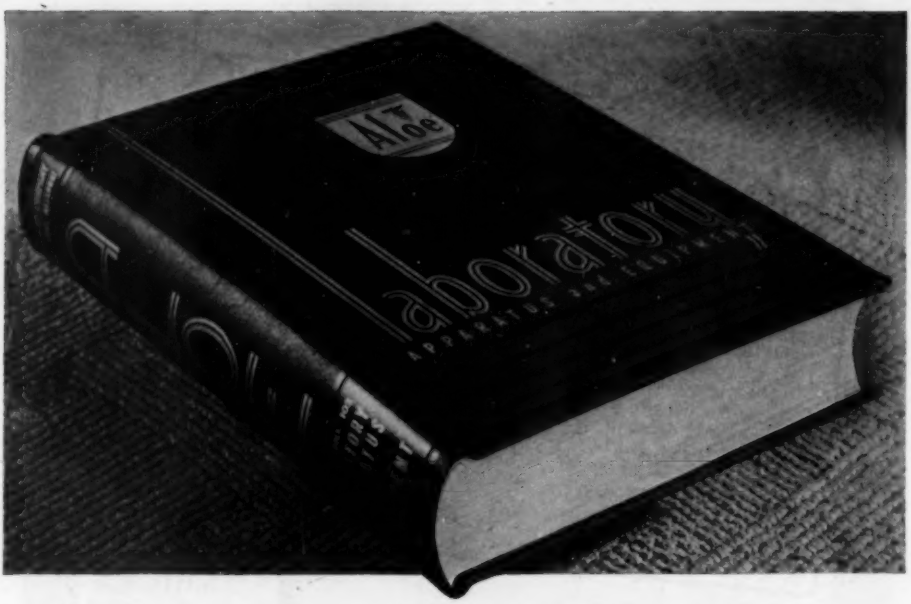

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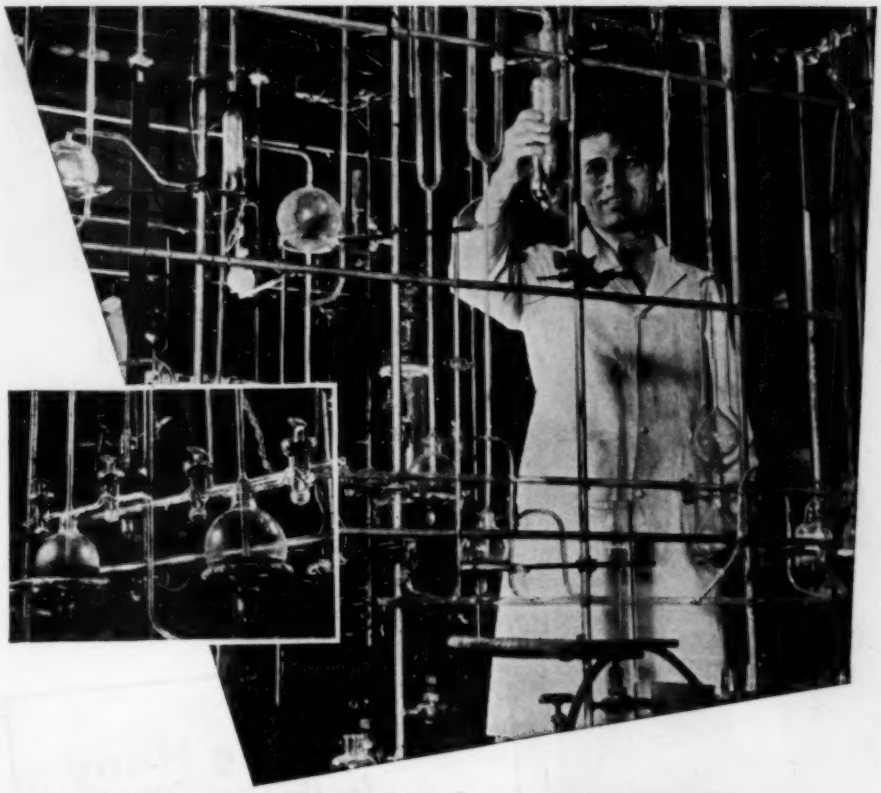

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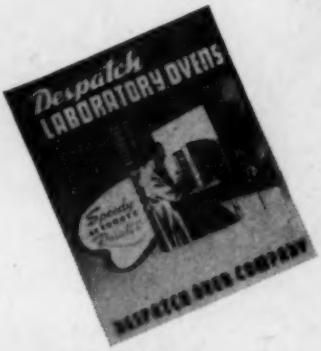

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